

How to Fix Tranquillizer Prices

THE British government has probably stirred up more trouble than it can reasonably have expected by its decision, three weeks ago, to order Hoffmann-La Roche to reduce the prices charged to the National Health Service for the tranquillizers called (in Britain) 'Librium' and 'Valium'. For one thing, there is likely to be a court case. The Swiss company and its British subsidiary are talking of taking the issue to the House of Lords, complaining that the Department of Trade and Industry has acted illegally under the terms of the Monopolies Act which does give the British government power to regulate the prices charged to public authorities by monopoly suppliers. Nobody will dispute the truth of the findings of the subcommittee of the Monopolies Commission which, three weeks ago, argued that Hoffmann-La Roche has been making handsome profits from the sales of the two tranquillizers. The trouble is that there is no easy rule of thumb for telling when handsome profits are what the government now calls "excessive". Another difficulty is that the fact that the two tranquillizers in dispute account, on various estimates, for something like 63 per cent of the tranquillizer market in Britain, owes at least something to their cheapness compared with other comparable drugs. It may be true that Hoffmann-La Roche has promoted its products vigorously, but it is also true that two-thirds of the patent protection it has enjoyed has now expired. Whether the case goes to the House of Lords, and whatever may be the outcome, there is at least a strong case for thinking that the government would have been wiser to respond to the report of the Monopolies Commission by taking action under the patent legislation than by dubiously enforcing one of the provisions of the Monopolies Act. At the very least, it is unseemly that a monopoly customer should use its own legislation to enforce a chosen pattern of behaviour on a somewhat less monopolistic supplier.

This said, it is very much to be hoped that the Hoffmann-La Roche case will not be allowed to sink into oblivion. To begin with, it brings sharply to the surface the question of how governments such as the British, now a member of the European Community, are to deal with what used to be called multinational companies but which are now European in some strict sense (for Switzerland is not, after all, that far from Brussels). In many ways, the issue of how Hoffmann-La Roche should conduct itself is a better precedent for the good conduct of European multinational companies than the recent decision of the European court and the expansionist proclivities of the Continental Can Company, an essentially American organization which has nevertheless been allowed to establish strong manufacturing organizations in Europe because there is no basis in equity for saying otherwise. In other words, it is to be hoped that if Hoffmann-La Roche gets no joy in the House of Lords,

it will have a try in the European court. What governments like the British have to decide is where they stand on the relationship between governments and private enterprise.

Pharmaceutical manufacturing is by any reckoning a speculative business, not much different from the business of drilling holes in the ground in the hope of finding gold. Pharmaceutical manufacturers are inclined to imply that their speculative expenditure is sanctified by the name research, and that it is therefore more virtuous than what people in Texas call "wildcatting". But there is not very much difference, and indeed the willingness of pharmaceutical companies to invest large sums of money in the development of new drugs is likely to persist for so long as those who need or use drugs consider it to be inestimably valuable to have something that performs better, even a tiny bit better, than what exists at present. In circumstances like these, the British government, which has fired the first shot at Hoffmann-La Roche, ought at the very least to be asking itself whether it should not now take action against all those entrepreneurs who have in the past few years bought tiny plots of land and found them appreciating enormously in value, who have invented new devices for helping to make computer systems work more efficiently and then found themselves rich beyond their expectations. For what the government has done over 'Librium' and 'Valium' is to say that even in a strictly speculative field of enterprise, profits must never be immoderate.

It would be much more sensible if the British government were to take a simpler and more radical view of the problem which confronts it. For practical purposes, the National Health Service is a monopoly customer for drugs, and the British government is well placed to act as a customer on the international market, seeking out the drugs which the National Health Service needs, making arrangements with the patent holders to manufacture under licence, and then arranging to manufacture. There is no valid reason why the National Health Service should not be somehow closely involved with the machinery for providing itself with essential supplies except that successive governments have always fought shy of anything that smacks of interference either with the medical profession, pharmaceutical manufacturing or the high street chemists. But the truth is that the National Health Service and the British taxpayer might have had a much better deal if the government had recognized its responsibility in this field and had taken a firm grip on the machinery by which drugs are at present supplied. What this implies is that the arbitrary action against Hoffmann-La Roche, populist though it may be, is not even the first step towards the solution of the problem which the government must sooner or later tackle.



Burn More Coal

THE British government has quickly echoed the United States government with a modest response to the increasing scarcity and price of petroleum. First of all, it has been agreed that the Central Electricity Generating Board will in the coming year burn more coal in British power stations than would be dictated by simple considerations of price. To make sure that British power stations do not turn to petroleum, at present prices a somewhat cheaper fuel than coal, the government has agreed to pay a subsidy of £1.75 a ton for the extra coal burned at power stations in the coming year. The decision is meant to cover only a limited period of time, for plainly there is at least a chance that the growing American demand for Middle East petroleum and the increasing toughness of the oil producing countries will bring about further increases in the price of petroleum in the coming year, so that a much smaller subsidy, or perhaps even none at all, will be appropriate in 1974. Whatever the future may hold, the decision is, however, a great convenience for the coal industry itself—without a subsidy for power station coal, there would have had to be a further contraction of coal mining in Britain in the months ahead, and the British government is well aware that each million tons of coal represents the jobs of 2,000 people under British conditions. In other words, the coal subsidy is as much a part of the British government's new attitude towards industries and groups of workers in trouble as of a deliberate energy policy as such.

It is all very well for the British government to live from hand to mouth in ways like these—indeed there is some virtue in a pragmatic approach to the problem of how to ensure adequate supplies of fuel in the years ahead. At the very least, the government needs some way of anticipating the consequences of sharp increases in the price of petroleum in the months ahead. It would, of course, be a great waste of resources to be shutting down coal mining operations this year and then paying prices for petroleum next year in excess of what coal would have cost. In other words, if the subsidy for the extra coal now to be burned in power stations is simply a bridging operation, there is no objection to it. The danger is that it will become sanctified with the passage of time and converted into a general undertaking that the British government will support the British coal industry at the level to which the National Coal Board and the country's coal miners are now accustomed. And the truth, of course, is that in spite of all the considerable improvements of technical efficiency, and the increases of productivity which have resulted, British coal is still comparatively expensive. Certainly it is an exceedingly costly commodity alongside, say, coal from the productive coal seams of the United States. Moreover, coal mining remains a relatively labour intensive industry, which implies that the real cost of coal is bound to increase more quickly than the prices of other commodities as real incomes increase. This is why the British government should not be alarmed by the fear of increased American competition for Middle East oil and rapacity on the part of OPEC countries into a policy involving a minimization of purchases of petroleum from the Middle East. After all, in the next few years it is entirely possible that the position of the OPEC countries will be turned by

the discovery of substantial deposits of petroleum elsewhere than in the Middle East.

At the same time, it is fair to say that the British government, like the United States Administration a week ago, has said too little about its plans for the support of research and development in other fields of energy supply. President Nixon, to be sure, did say in his energy message to Congress that expenditure on thermonuclear fusion would be increased, and this is entirely appropriate. It is welcome that the corresponding British programme is likely also to be more fully supported in the months ahead. But is there not a case for a much more closely coordinated and sharply pointed programme of research and development on breeder reactors, which are bound to have to be, in the 1980s and the 1990s, the work horses of nuclear energy? And is it not high time that some of the more futuristic schemes for extracting energy from, for example, the surface layers of the oceans which are heated by solar radiation, were investigated properly?

100 Years Ago



A VOICE FROM CAMBRIDGE

IT is known to all the world that science is all but dead in England. By science, of course, we mean that searching after new knowledge which is its own reward, a thing about as different as a thing can be from that other kind of science, which is now not only fashionable, but splendidly lucrative—that “science” which Mr. Gladstone and Mr. Lowe always appeal to with so much pride at the annual dinner of the Civil Engineers—and that other “science” prepared for Jury consumption and the like.

It is also known that science is perhaps dearest of all at our Universities. Let any one compare Cambridge, for instance, with any German university; nay, with even some provincial offshoots of the University in France. In the one case he will find a wealth of things that are not scientific, and not a laboratory to work in; in the other he will find science taking its proper place in the university teaching, and, in three cases out of four, men working in various properly appointed laboratories, which men are known by their works all over the world.

This, then, is the present position of Cambridge after a long self-administration of the enormous funds which have been so long accumulating there for the advancement of learning. Cambridge no longer holds the place which is hers by right in the van of English science, her workers are few, and to those few she is careful to afford no opportunity of work, such as it is the pride of scholastic bodies in other countries to provide for the men who bring the only lasting honour to a university.

From Nature, 8, 21, May 8, 1873.

OLD WORLD

ELDO Ends Europa II Programme

THE European Launcher Development Organization (ELDO) has abandoned its work on the Europa II launcher. The ELDO council was left with no alternative but to terminate the project following an announcement by France and Germany that they would not be able to support it from May 1. As most of the finance comes from these two countries the council directed the Secretary General of the organization to close down the projects and inform those companies which hold contracts from ELDO that the programme has ended.

The decision leaves the 9-year-old organization with no current programme and no immediate prospect of one, although its legal framework still exists.

The closure of the programme follows the cancellation of the Europa III project last year and the failure of F11, the first test firing of Europa II in November 1971. In all, ELDO has launched eleven rockets, only six of which have been completely successful, chiefly in the early stages of the development of Europa I.

Precisely what will happen to the organization is not yet clear. ELDO employs a few more than 300 people in Paris and at Kourou in French Guiana, ELDO's test centre, some of whom are certain to be made redundant. The member countries of ELDO will have to decide what is to happen to the organization, and the likeliest possibility at present is that it will be restructured with the intention of retaining those personnel with expertise in launcher development, and organizing the liquidation of the Europa project. But quite what, if anything, the organization will do between now and January 1, 1974, when it is to be merged with the European Space Research Organization in a European Space Agency is not clear (see *Nature*, 240, 516; 1972).

The decision to end the Europa programme leaves Europe with no plans to develop a launcher other than those of the French to build the L3S rocket. This was proposed as the alternative to Europa III and approved in principle by the European Space Conference last December, although no funds other than from France have yet been put into it. It is possible, if the French can persuade the Germans to participate, that the new agency will develop L3S as a cooperative venture, using some of the expertise assembled within ELDO.

But there are even doubts about the

continued viability of L3S for, although there is some provision for developing the launcher in the French budget this year, enthusiasm for the project may have waned somewhat now that M. Michel Debré, the launcher's chief protagonist, is no longer Minister of Defence following the French general elections.

The decision to end the Europa project typifies the continuing disagreement within Europe on the subject of launchers. Germany (and Britain) have long advocated buying launchers from the United States as and when they are needed. The French, on the other hand, argue that Europe must maintain an independent capability, and it is this that led to the proposal to develop LS3, a launcher with similar capabilities to Europa III but which, the French claim, could be built at about two-thirds of the cost.

But whether L3S is developed or not, the French, the Germans, and the

European Space Research Organization are all going to have to rely on the United States to launch *Symphonie*, the Franco-German communications satellite, and COS-B, ESRO's next scientific satellite, both of which were due to be launched on Europa II.

DRUGS

Safety or Service?

CONCERN over the safety of new drugs must not be allowed to get out of proportion, according to Mr Ivar Boden, retiring president of the Association of the British Pharmaceutical Industry. Mr Boden, speaking at the annual dinner of the association recently, said that it is unlikely that entry into the European Economic Community will markedly change the strategy of the chief British drug companies. He hoped that Britain would not be forced to change its already well established system for

ROYAL INSTITUTION

Healthier Situation

IN spite of grave predictions made a year ago the Royal Institution showed a slight profit for its operations during 1972. When the 1971 annual report came out showing a deficit of £7,812, the then newly appointed treasurer, Sir Gordon Cox, forecast a deficit of £17,600 in 1972. But economies in nearly all the Royal Institution's operations combined with a few benefactions resulted in a profit of £5,486 last year.

The figures for 1971 and 1972, however, are not directly comparable because a new system of classifying the accounts and a new system of treating the money available for research have been introduced. The net result is that the Royal Institution is more than £16,000 better off this year than last.

The process of selling off books which were not considered essential has now been concluded and £150,000 has been raised which has been invested for the benefit of the library. The interest from this money was £8,575 in 1972 and more will be expected this year from this source.

The membership drive, which was expected to increase the membership by 300 a year, failed to reach this target because 50 members either died or did not renew their subscriptions—the net increase was 250.

One of Sir Gordon's aims is to make the membership activities self sustaining

—last year there was a deficit of £3,268 in this sector. But the trend is most satisfactory and it is hoped that these activities should, at least, break even during 1973.

Apart from economies in most activities, one of the other reasons for the accounts looking much more healthy in 1973 is an anonymous gift of \$188,000 over ten years to be used to provide administrative assistance and services for the director, Sir George Porter. In 1972 this fund added £5,846 to the accounts. Also during 1972, Applied Photophysics Limited contributed £4,027 to the Royal Institution.

But what of the future? During the past ten years the Royal Institution has accumulated a deficit of about £50,000. Sir Gordon Cox said this week that it is his intention to make good that sum in the next ten years. But because of inflation, twice this amount will have to be recouped before the finances are in the same situation as they were in 1963.

As far as 1973 is concerned several effects make predicting the financial position at the end of the year uncertain. First, the effect of corporation tax is unknown, and Value Added Tax will take about £2,000 of the Royal Institution's funds. Because of the changed system of income tax in Britain the institution will probably suffer a decrease in income, estimated to be about £500, from recovered income tax covenants.

licensing medicines, and he emphasized that the industry must still regard the possible dangers of new drugs with the utmost concern.

But while products should continue to be manufactured with safety very much in mind, Mr Boden said, safety must not become too much of a threat to the progress of science. If this happens it will take much longer to pass on the benefits of research to society.

Those who are demanding that new drugs be absolutely safe before release to the public are pursuing a myth, according to Mr Boden. The cry of safety at all costs is impractical and the cost of research must not be raised to a level that no manufacturer can afford.

There are signs that pressure is being brought to bear to slow down the work of the Committee on Safety of Medicines, and it would be sad, said Mr Boden, if the British pharmaceutical companies had to look outside this committee for somewhere to carry out early clinical trials on drugs.

People must, of course, have a right to obtain adequate compensation for injury sustained from drugs, Mr Boden said, but the sum of nearly £1 million a day that is spent world wide in research by the pharmaceutical industry must be used to optimum advantage.

SPACE

Copernicus-500

THE joint Polish-Soviet solar physics research satellite, promised last February as part of the Copernicus quincentenary celebration, was launched on April 19 as part of the Interkosmos series. The satellite is designed to study "sporadic" solar radiation, in particular the radio emission from the Sun between 0.6 and 6.0 MHz and also to investigate the characteristics of the ionosphere. Its orbital parameters are: perigee 202 km, apogee 1,551 km, inclination 102.2°.

"Copernicus-500", as this special Interkosmos is called, carries low-frequency and high-frequency ionosphere probes of Soviet manufacture, and a radiospectrograph developed by a Polish team based in Toruń, the birthplace of Copernicus. This is the first time that this Polish instrument has been used in the Interkosmos programme, although similar radiospectrographs have been carried by the Comecon high-altitude geophysical probes.

Interviewed in *Pravda*, the head of the Polish side of the Copernicus project, Academician Dionizy Smolenski, spoke of the particular contributions which Poland hopes to make to the Comecon space projects. Four chief fields are involved: space physics (including space geodesy), meteorology, communications and space biology and

medicine. In particular, Polish scientists are playing a considerable role in the *Dinamika* programme which comprises a number of experiments on the Earth's gravitational field, undertaking the co-ordination of theoretical and experimental work on the improvement of methods of observing and computing the trajectories of space objects for geodesic purposes.

Though "Copernicus-500" was announced as a joint Polish-Soviet experiment, the other Comecon countries are also participating. Czechoslovakia has provided a special on-board telemetry transmitter, and synchronized solar observations are being made from astrophysical and geophysical observatories throughout the Comecon block.

EDUCATION

Social Significance

from a Correspondent

AN association to further the social significance of the teaching of science and mathematics in the British Commonwealth has been set up as a result of a meeting of representatives of seventeen countries which was held at the University of the West Indies at Kingston, Jamaica, last month.

The acting chairman of the association (Commonwealth Association of Science and Mathematics Educators, CASME) is Mr Maurice Goldsmith, chairman of the Guinness awards, which organized the symposium.

One of the aims of the association is to ensure that the natural sciences, technology, mathematics and the social sciences are integrated in the training of teachers. This, however, requires that teachers must be trained in a different way. CASME will consider, among other topics, how this can best be done.

LUBRICATION

Contract for Swansea

THE Tribology Centre at the University College of Swansea has been awarded a contract by the European Space Research and Technology Centre to look into the use of molybdenum disulphide as a lubricant. Although the compound has been used in the United States' space programme, for example to lubricate the legs of the Apollo lunar modules, European designers have been reluctant to use it because there is a conflict in the literature about its suitability in space conditions.

Dr A. R. Lansdown, Director of the Tribology Centre said this week that although the contract is a preliminary one worth less than £10,000 he is hopeful that it will be followed by others.

NATURE CONSERVANCY

New Council Chairman

SIR David Serpell, former Permanent Secretary at the Department of the Environment, is to be the first chairman of the new Nature Conservancy Council which is due to come into existence shortly.

The new council, first outlined in last July's white paper on Government research and development, will take over part of the functions of the Nature Conservancy, and will be responsible for establishing, maintaining and managing nature reserves in Britain, and commissioning and supporting relevant research. The council will have a budget of about £1.4 million, £0.3 million of which will be spent on research. The remainder of the Nature Conservancy's work will remain within the Natural Environment Research Council, of which the conservancy is a part, and a new Institute of Terrestrial Ecology will be formed by the council.



Sir David Serpell

Sir David, 61, who retired in September last year, has been a civil servant for more than thirty years, working at various times for the Ministries of Food, Fuel and Power, and Transport, with spells at the Treasury and the Board of Trade. In 1970 when the Department of the Environment was formed, he became its first permanent secretary. A graduate of the University of Oxford, the University of Toulouse and Syracuse University in the United States, he is also a fellow of the Fletcher School of Law and Diplomacy in the USA.

NEW WORLD

Public Accountability and the Academy of Sciences

by our Washington Correspondent

THE National Academy of Sciences, the most prestigious scientific organization in the United States, was sharply criticized last week for allowing some of the committees which advise the government in its name to be "subverted by special interests". The charges were made in a speech delivered at the annual meeting of the American Physical Society by Philip M. Boffey, who for the past two years has been conducting an investigation of the academy for the consumer advocate Ralph Nader. Academy officials have responded to Boffey's charges by suggesting that they are aware of the problems to which he referred and that steps have already been taken to overcome them.

Unlike the Royal Society of London, whose chief purpose in life is to elect new members, the National Academy of Sciences is perhaps the most important source of supposedly independent scientific advice to the federal government and thus charges of bias are not to be taken lightly. The process by which the academy advises the government works like this: Federal agencies which need help with a problem contract with the academy for advice, a group of experts (not necessarily academy members) is appointed through the National Research Council—the Academy's operating arm—and ultimately a report is produced for the contracting agency. At a recent count, some 8,300 scientists were advising the government through academy committees on problems ranging from the SST to the use of chicken feathers to stuff army mattresses.

Boffey told the American Physical Society that two chief themes have emerged from his study, namely that some academy committees "fall captive to the thinking of the government agencies which contract for their services", while others sometimes "fall prey to industrial interests". He illustrated these charges with examples of the workings of two academy committees, drawn from several case studies which will be documented in his final report to be published later this year.

Boffey suggested in his speech, and also in an interview afterwards, a number of situations in which the academy can unwittingly become aligned with the agency which contracts for its services. First, there is the old Washington trick of appointing a prestigious study group as a means of heading off

public criticism of a programme. Second, he suggests that academy committees are sometimes inclined to pull their punches so that they do not lose future contracts with that particular agency. And finally, he maintained that the practice of showing draft reports to the agency before final publication "has a subtle eroding effect on the committee's ability to be critical".

As illustration, he pointed in his speech to a committee set up in 1964 to study the possible environmental problems associated with supersonic transporters. The committee was set up under contract with the Federal Aviation Agency in the aftermath of public opposition to sonic boom tests in Oklahoma, and the committee's first report "incredibly . . . recommended that the government conduct a public relations campaign to persuade people to accept the SST". The conclusion of

a 1968 report from the committee that "the probability of material damage being caused by sonic booms generated by aircraft operating in a safe, normal manner is very small" eventually led 189 academy members to sign petitions contending that the report was inaccurate and should be corrected. No such correction was made public, however, and similarly a note produced by the committee "clarifying" its report and qualifying its chief conclusion was also never publicly issued.

As for bias towards industry, Boffey chose as an example the Food Protection Committee, which has already had a rough time at the hands of critics in testimony to Congress and in newspaper articles. The committee and its subpanels, Boffey said, "reflect a consistent bias—they are lenient about recommending action that might restrict the use of chemical additives in the

NATIONAL ACADEMIES

Divorce Imminent

by our Washington Correspondent

FOR the past nine years, the National Academy of Sciences and the National Academy of Engineering have been on the verge of divorce, but the split now seems about to be made final. The membership of the National Academy of Engineering is being asked by the academy's governing council to vote this week on a resolution that the NAE be incorporated independently of the NAS and that a foundation be set up to raise money to enable the NAE to carry on its work of advising the government on questions of engineering and public policy. The membership must agree to the resolution at meetings held on May 3 and October 24, before the divorce becomes final.

The split ends nine years of fruitless efforts by the councils of the two academies to work out a mechanism to allow the academies to share in the governance of the National Research Council, and the divorce is being greeted with mixed feelings. Set up in 1964, the NAE has been operating under the charter of the NAS, and the original intention was that the two academies would work together in giving advice to the executive branch of the federal government. But the NAE has set up its own network of advisory committees instead of working through the National Research

Council, partly because the control of the research council is vested solely in the hands of the council of the NAS.

A part of the problem has been mutual distrust between the two academies, with NAS members being distrustful of conflicts of interest that arise when the corporate executives who are members of the NAE serve on advisory committees, and members of the NAE being unwilling to work in an organization whose management is out of their hands. (And the distrust felt by members of the NAS has been sharpened recently by the election of Robert Seamans Jr., secretary of the Air Force, as President of the NAE. Seamans, who will step down from his government post before assuming the presidency, is regarded by his critics as an integral part of the military-industrial complex.)

Although there will be members of both academies who welcome the divorce, it is a keen blow to attempts to keep the NAS a multidisciplinary body able to offer advice on broad social questions. The NAS annual meeting passed a resolution last week, however, extending an invitation to the NAE "to work together to develop effective means for the two academies to cooperate in the national interest on activities of mutual and overlapping concern". Clarence H. Linder, the present president of the NAE has already stated his hope that the two academies will be able to work together.

food supply and they downplay the likelihood of such long-term hazards as cancer, genetic damage and birth defects".

He suggests two chief reasons for the food protection committee's alleged bias. First, it has close ties to industry through its funding—in 1972, 40 per cent of the committee's budget was provided by sources in industry—and through an industry liaison panel which gives industry "a privileged voice in committee affairs". And second, the committee's panels have seldom contained experts in mutagenesis, teratogenesis or carcinogenesis.

Asked last week for his comments on Boffey's charges, Dr Philip Handler, President of the National Academy of Sciences, called his speech "the greatest accolade we have received yet", the rationale behind that remarkable conclusion being that the two examples chosen by Boffey are well known and that the academy has already taken steps to deal with such problems. Handler argued that the academy has about 500 committees and if Boffey can only dig up those two "old chestnuts", there cannot be too many skeletons in the cupboard. Boffey points out, however, that those two examples were drawn from many which will appear in the final report.

Handler also drew attention to several reforms which the academy has recently instituted in an attempt to eliminate bias from its committees and to prevent the committees becoming too cosy with the contracting agencies. From January this year, for example, every committee member must sign a statement giving details of employment and consultancies in the past 10 years, research support during the past five, current financial interests and any publicly stated positions on the subject matter before the committee. To help overcome the problem of committees becoming aligned with the contracting agency, Handler pointed out that the academy has instituted a rule that members are appointed for fixed terms of three or four years and that reappointments must be closely justified.

But perhaps the chief reform instituted in recent years has been the setting up of a Report Review Committee under the chairmanship of Dr George Kistiakowski. The committee examines each report issued in the academy's name for scientific accuracy, possible bias and ambiguous phrasing, and suggests changes both to the committee concerned and to Handler. The committee consists of academy members.

But Boffey suggested that although such reforms are "worthwhile palliatives to the basic problems, they are by no means the complete answer", and likened the process to that of car manufacturers cleaning up exhaust emissions

by fitting add-on catalysts instead of developing a non-polluting engine. As for bias statements, Boffey pointed out that many members of panels of the Food Protection Committee have come under attack for potential sources of bias but they are still serving.

The provision that members of committees should serve for fixed terms is a "totally inadequate response" to the problems of committees becoming too cosy with the contracting agency, Boffey suggested. It does not, for example, attack the problem of staff members of the academy being anxious to preserve contracts with the agencies, and Boffey said that he has "evidence of subservience to the executive branch as of two days ago".

As for the report review committee, Boffey says that "is one of the best things that has happened to the academy in recent years. It is a big step in the right direction". But even so, he believes that the committee could be made much more effective if its reports and comments were made public. He cited in his speech an example of a report by the Food Protection Committee on *Guidelines for estimating toxicologically insignificant levels of Chemicals in Foods* which was savagely attacked by a group of cancer experts, whose views were acceptable to the review committee. But the review committee's comments have never seen the light of day, and the academy is still in the position of seeming to support recommendations with which one of its most distinguished committees disagrees.

Essentially what Boffey is asking for is more public accountability in the academy. His study, the first independent investigation of the academy since it was founded during Lincoln's presidency in 1863, may help push the academy in that direction.

AMERICAN PHYSICAL SOCIETY

Pyrrhic Defeat

by our Washington Correspondent

THE membership of the American Physical Society has rejected a proposed amendment to the society's constitution which would have added to the present aims of the society, which are simply "the advancement and diffusion of the knowledge of physics", the words "in order to contribute to the enhancement of the quality of life for all people. The society shall assist its members in the pursuit of these humane goals and it shall shun those activities which are judged to contribute harmfully to the quality of mankind". Proposed by Robert H. March, a nuclear physicist from the University of Wisconsin, the amendment was defeated by 45,000 votes to 37,000.

March said last week that he is neither surprised nor disappointed at the vote, because the resolution has essentially become redundant. The society is already engaged in discussions of social responsibility through its newly-established Forum on Physics and Society, he said, and the council has come a long way during the past year towards agreeing with the views of several supporters of the amendment who simply wanted such issues debated. March himself was unhappy about the wording of the last part of the amendment, which has borne the chief brunt of the criticism, and at one stage was prepared to drop the part instructing the society to shun some kinds of activities (see *Nature*, **240**, 518; 1972). Legal difficulties prevented alteration of the proposed amendment, however.

Short Notes

Classified Research

Dr Edward Teller, so-called father of the US H-bomb, last week urged Congress to pass a law requiring all secret government scientific research to be declassified a year after it is completed. Speaking at the annual meeting of the American Physical Society, Teller argued that secrecy hinders the progress of science and urged government officials to pay more regard to the damage resulting from overclassification than to the damage likely to result from making research results public. He also suggested that the efficiency of intelligence operations makes classification pointless for longer than a year, and included weapons design in his definition of scientific research.

DES Banned Again

When the Food and Drug Administration banned the use of the synthetic hormone diethylstilboestrol (DES) in animal feeds last year, it did not prevent farmers from implanting pellets of the hormone in the ears of cattle. Used as a growth promoter, DES produces the same effect when used as an ear implant as when added to cattle feeds. But last week, the FDA banned that use as well, making the ban on DES as an agricultural chemical complete. The hormone was taken off the market as a feed additive last year because radioactive tracer techniques showed that it remained in beef liver for up to 120 days, and thus could not be prevented from turning up in the meat supply. Since the hormone is highly carcinogenic, the FDA had no alternative but to ban it. The same sensitive tracer studies have now been applied to ear implants, and not surprisingly they have turned up the same results, hence the ban last week.

NEWS AND VIEWS

Going in the Red

RHODOPSIN, the visual pigment of retinal rods, is a molecule that has caused many a theoretician to shed tears of bitter frustration. Its chemistry, thanks largely to the work of Wald and his colleagues, is understood in its essential details, but its absorption spectrum is all but unexplained. When the carotenoid prosthetic group, 11-*cis* retinal, which is pale yellow, is bound to the protein, opsin, an enormous spectral shift ensues, with the appearance of a red, or in the case of the cone pigment, iodopsin, a purple colour. To account for such a formidable wavelength shift—200 nm or even more—has taxed even the fertile imagination of the spectroscopists. There is good evidence that the terminal aldehyde group of the retinal is bound by way of a Schiff base linkage to the amino group of a lysine side chain in the protein, and an increase in the length of the π -electron system, with a consequent redshift in the absorption band, would result if a protonated aldimine group were added to the end of the carotenoid system.

Now the electronic spectra of the carotenoids are as well understood as anything in molecular spectroscopy, and certain it is that the extension of the π -system by this much can explain only a small part of the observed shift, as indeed is also experimentally obvious from the spectra of model compounds. Explanations of the spectra of the visual pigments, which directly determine the wavelength-dependence of the visual response, have therefore tended to invoke perturbations, unknown to science in any other context, involving special dispositions of charged groups around the chromophore, massive induced dipoles, and so forth. After all these years of whistling in the dark, an altogether more convincing hypothesis has at last appeared. This is propounded by Mendelsohn, writing in this issue of *Nature* (page 22), and with hindsight, it seems perhaps slightly surprising that this perception has not come sooner.

Mendelsohn has not actually worked with a retina pigment, but rather with the closely related material from the purple membrane of the halophilic bacterium, *Halobacterium halobium*. The chromophore, as in rhodopsin and iodopsin, is 11-*cis* retinal; it has been shown to be attached to the protein through a Schiff base with a lysine amino group, and its absorption spectrum is in essence that of a visual pigment, for which it may therefore be regarded as an analogue. The advantage of this material is that it is much more stable to light, and is not in fact photochemically bleached. This makes it a promising object for study by the resonance Raman technique, which, it has been lately pointed out, offers certain unique advantages in regard to chromophoric biological systems. The principle is that when the exciting line falls in the electronic absorption band, and there are no complications from fluorescence or photochemical lability, Raman-active frequencies of the chromophore are greatly enhanced in

intensity, and the spectrum can therefore be examined at very low concentration, and without interference from the contributions of other species, the intensities of which are by comparison infinitesimal.

Thus laser excitation within the absorption band of the purple membrane leads at very low total pigment concentration to a Raman spectrum containing some fifteen peaks, which vanish if the pigment is first bleached by denaturation with cationic detergent. The most prominent band lies at $1,531\text{ cm}^{-1}$ and its relative intensity increases as the excitation wavelength approaches the visible absorption maximum. A second intense band at $1,568\text{ cm}^{-1}$, on the other hand, becomes more intense when the excitation wavelength shifts towards the blue. It is inferred that the two vibrations are coupled respectively to the longest-wavelength electronic absorption band, and to a transition at shorter wavelength. All other vibrations behave like the $1,531\text{ cm}^{-1}$ band. With the aid of published assignments for the Raman spectra of retinal derivatives, the principal features in the resonance Raman spectrum can be identified, in particular those in the region containing C=C and C=N stretching modes. Comparison of the observed frequencies and their dependence on deuteration, with those of model compounds, makes it clear in the first instance that the chromophore contains an unprotonated Schiff base, which at once annihilates a widely held hypothesis. The most striking observation, however, is that the dominant $1,531\text{ cm}^{-1}$ band has no counterpart in the model compounds, and is surmised therefore to arise from perturbation of the π -electron system with some other element of the protein. Moreover the system is highly sensitive to solvent: addition of chloroform induces a reversible shift of 60 nm in the electronic absorption band, with a corresponding displacement of the intense Raman line.

All this is diagnostic of a charge-transfer interaction, and the best candidate for an electron donor in the protein is, as Szent-Györgyi perceived many years ago, the indole chromophore of tryptophan. Retinal and tryptophan when mixed in solution do indeed give rise to a new long-wavelength absorption band, indicative of the formation of a charge-transfer complex. The conclusion that the absorption band of the visual pigment arises in this way has yet, of course, to be proved, but its attractions and the several hitherto perplexing features of the system for which it at once provides an explanation render it a compellingly attractive proposition. It may moreover give pleasure to those spectroscopists who rallied fifteen years ago to Szent-Györgyi's banner, and set off in pursuit of Submolecular Biology, for it is not often that a charge-transfer complex turns up in nature, without a sprinkling of chloranil or iodine from the hand of the Master.

From our Molecular Biology Correspondent

Paradoxical Effect of ALS

RECENT advances in cellular immunology seem to have provided an understanding of the array of cells involved in immunological responses. It seems that many immune responses involve the participation of at least three kinds of cell—T lymphocytes of thymic origin, B lymphocytes of bone-marrow or bursal equivalent origin and macrophages. In some situations it is possible to deduce that the activities of T cells lead to the production of humoral factors which, when bound to the macrophages, can stimulate B cells to produce antibody. The net results thus seem to be attributable to the activities of an immunological orchestra. It may also prove to be the case that the responses to some kinds of antigenic stimulus involve only small sections of the orchestra. Clearly it will often be a complicated matter to gain a concise interpretation of the effects of an extrinsic immunosuppressant on such an intrinsically complex function. A report by Cantor and Asofsky on page 39 of this issue exemplifies this.

Antilymphocyte antiserum (ALS) has been found to be a potent immunosuppressive agent in a variety of animals. It is often supposed that its effect is caused by selective depletion of T cells and that it has little influence on the thymus as a source organ. There are, however, reports which have indicated an effect on bone marrow, on the thymus and on macrophages and thus any simplistic view

of the mode of action of ALS is probably wrong. The variety of results can in part be attributed to the variety of methods used to produce ALS and the variety of the producing animals. Cantor and Asofsky dealt with ALS produced according to a recipe of Levey and Medawar (*Proc. US Nat. Acad. Sci.*, **56**, 1130; 1966). They injected this material, in relatively small amounts, into parental mice. At various times later the capacity of the thymuses of these animals to engender a graft-versus-host (GVH) reaction in newborn F_1 recipients was determined.

Cantor and Asofsky found that the GVH activity of the thymocytes of recipients of ALS was much increased for about the same time that the comparable activity of the spleens of the same animals was reduced. There was no evidence of a reduction in the cellularity of the thymuses of the ALS-treated mice but no histopathological evaluation is given. Cantor and Asofsky do not feel that the effect they observe on thymuses can be attributed to stress which might reduce the cortical cell population as a result of high amounts of corticosteroid. They prefer to suggest that ALS somehow causes an alteration in the proportion of mature cells which are about to leave the thymus to become T cells. It is reasonably supposed that T cells initiate GVH reactions.

Whatever the mechanism involved, these experiments suggest that studies on immunosuppressive agents should take into account changes in the source organs of immunologically reactive cells. From a Correspondent

How Fickle is the Finger of Fate?

It is sometimes difficult to know how seriously to take those who suggest that the constants of physics are really variables. The numbers game is fun, of course, and is undoubtedly an exercise which ranks among the better kinds of mental gymnastics for preventing premature decay of the grey matter. But is there really any practical point to it? As Noerdlinger points out, in introducing the latest version of this game, "there are grave doubts as to the meaning of saying that any dimensional constant changes, because presumably dimensional constants only express something about the sizes of the standards we use in measuring them". Nevertheless, Noerdlinger goes on to consider the implications of the 2.7 K background radiation for the constancy of Planck's constant h (*Phys. Rev. Lett.*, **30**, 761; 1973).

The latest bout of speculation about the constancy of h seems to have been sparked off by the suggestion of Bahcall and Salpeter that measurements of the dispersion of QSO light by prisms and by gratings could provide a measure of the fickleness of the constant (*Astrophys. J.*, **142**, 1677; 1965). Their idea is straightforward enough: because prisms essentially react to the energy of photons whereas gratings measure wavelength and $E=h\nu$, comparison of the two kinds of observation would show up changes in h since the QSO light started on its journey. Experiments to test this possibility have come up with negative results in every case. But still, these experiments have only finite accuracy, so the possibility of a variable h remains. Now Noerdlinger has come up with an alternative test which, while proving equally negative (or equally

positive, perhaps, if one's point of view is that universal constants really should be constant throughout time and space), does provide further mental stimulation.

A fundamental requirement of any such contribution to the numbers game is to make enough initial assumptions to ensure that something can be deduced. Noerdlinger's assumptions about the origin of the black-body background are reasonable, although one could quibble over his exact choice of parameters, and do not seriously affect his principal point. Like the QSO light idea, this is quite simple. The Rayleigh-Jeans part of the measured 2.7 K curve gives the present value of kT irrespective of the value of h . On the other hand, the turnover in the spectrum gives an indication of the parameter $(h\nu/kT)$. Treating kT as one parameter, and leaving aside any question that k changes, a measure of h which might be expected to carry some memory of conditions earlier in the evolution of the Universe again seems possible. If h was larger in the past, for example, the spectrum ought to turn over below the points measured. Noerdlinger concludes that $\delta h/h < 0.3$ in the interval of redshift $0 < z < 1,000$ (his upper limit on z corresponds to the assumed time at which the last energy interchange between matter and radiation occurred—the end of the decoupling era).

An interesting possibility remains, however. If the QSO experiments do produce a positive result, the lack of a positive result from the background radiation test might imply that the speed of light varies, that one should not write $(h\nu/kT)$ but rather $(hc/\lambda kT)$, and/or that the 2.7 K radiation is travelling at a speed not equal to c .

By our Cosmology Correspondent

SYSTEMATICS

Hope for Britain's Wildlife

from a Correspondent

ON April 11, 12 and 13 the Systematics Association held a symposium in Leicester to consider the changes which are occurring in the numbers and distribution of the principal groups of plants and animals in Britain. This was the first meeting with such a comprehensive cover since the Linnean Society's conference in 1935.

Professor K. Mellanby (Monks Wood Experimental Station and University of Leicester) opened the proceedings with a description of the environmental changes—climatic, population and industrial—with which the flora and fauna have to contend. He also summarized the general conclusions in the final contribution. Almost every speaker had used maps prepared by the Biological Records Centre at Monks Wood. The success of this centre depends on the help and collaboration it receives from many observers and recorders, professional scientists and amateurs alike. Professor Mellanby thought that this practical cooperation between conservationists augurs well for the future, and that it will encourage the scientists from the Nature Conservancy, who are being reorganized into the Institute of Terrestrial Ecology within the Natural Environment Research Council, to continue to direct their research into fields of wildlife conservation.

Dr F. H. Perring, also of Monks Wood, dealt with flowering plants. Some twenty species have disappeared since recording began, and fifty are classified as "endangered", but others, not all introductions, have extended their range considerably. Dr F. Rose (Kings College, London) dealt with bryophytes and Dr L. Hawksworth and Mr B. J. Coppins (Commonwealth Mycological Institute) with lichens. These groups have been used as delicate indicators of atmospheric pollution, and several species are endangered by such factors as tree felling and the general "tidying up" of old buildings and of the countryside generally. Dr W. E. Jones (University of Wales, Bangor) spoke on seaweeds, on which new studies by divers are extending knowledge.

Dr G. B. Corbet (British Museum (Natural History)) said that although six native species of mammal became extinct between Roman times and 1800, the remainder seemed to be holding their own. Dr J. T. R. Sharrock and Mr K. Williamson (British Trust for Ornithology) showed that there has been a net gain of five new species of bird entering Britain every decade since 1950, notwithstanding habitat destruction and human

persecution. This was a success story for practical conservation. Dr T. T. Macan (Freshwater Biological Association) and Mr A. Wheeler (British Museum (Natural History)) were both chiefly concerned with the success of introductions of invertebrates and fish to freshwater.

Mr I. Prestt (Department of the Environment) spoke on reptiles and amphibians, each of which have six British species. Several of these are really endangered, and the numbers of others, including the common frog, have decreased. Dr A. South (Sir John Cass College, London) showed that although some snails have suffered, some slugs are well adapted to modern agriculture and are now pests. The spores of pathogenic fungi are widespread according to Dr C. Booth (Commonwealth Mycological Institute, Kew), and records show the conditions which make their presence known (for example, disease outbreaks). Dr D. R. Reid (also from Kew) showed how fungal records are sometimes incomplete because collectors get up too late to see transient forms visible only soon after dawn.

Dr E. Duffey (Monks Wood) showed how records have improved, and the British list has lengthened, as a result of the recent efforts of the Arachnological Society. Only one spider is thought to have disappeared in recent years. Mr J. Heath (also of Monks Wood) described how habitat changes affect butterflies and moths, but showed that recently improvements in distribution often balance disappearances and the situation, generally, is not entirely unfavourable. In the cases of other insects, Mr J. C. Felton (Shell

Chemicals) and Mr K. G. V. Smith (British Museum (Natural History)) showed that, in the Hymenoptera and Diptera respectively, the species recorded increase yearly as studies are intensified. Mr Smith indicated that man is deliberately trying to exterminate flies which bite man. Professor Mellanby described how some human ectoparasites are faring; head lice are getting commoner because habitat improvement (longer hair) favours the insects.

The chief impression gained from the Leicester symposium was that although some species and many habitats are endangered by population, affluence, mobility and industrial growth, the position is not nearly as bad as is often suggested. The report of the World Wildlife Fund a few days earlier had given a quite different impression, stating categorically that "nearly a quarter of all Britain's vertebrate animals and plants are threatened" and listing, as seriously endangered species, many which are well known to be flourishing and increasing. At Leicester the speakers were recognized authorities on their groups. Though generally reasonably optimistic, they were in no way complacent, and frequently drew attention to dangers, though they all avoided the hysterical and counter-productive attitude unfortunately characteristic of some "ecologists" and, now, of the World Wildlife Fund.

PALAEOICHTHYOLOGY

Tetrapod Ancestry

from a Correspondent

THE lobe-finned fishes (rhhipidistians) have long been regarded as the closest relatives of the tetrapods. Jarvik, who has worked on these fishes for almost

Nucleolar Single Stranded DNA

IN eukaryotic cells, as in *Escherichia coli*, newly replicated DNA can be extracted in a single stranded form and it has been suggested that replication of nucleolar DNA may be independent of the replication of the bulk of the genomic DNA of eukaryotes. These considerations led Almaric *et al.* to investigate the form of DNA that can be extracted from the nucleoli of ascites tumour cells and, as they report in *Nature New Biology* next Wednesday (May 9), single stranded DNA can indeed be extracted from the nucleoli of these tumour cells with hot phenol and cresol in the presence of para-aminosalicylate.

This single stranded nucleolar RNA does not, however, have the properties of newly replicated DNA; it remains constant for long periods of chase in

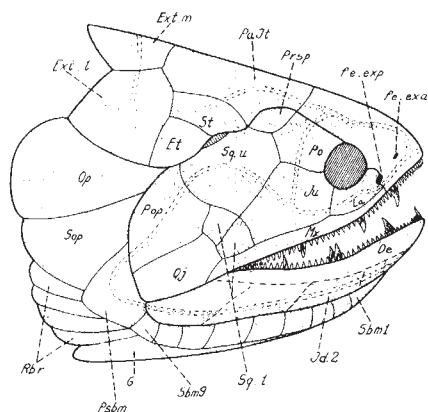
pulse chase experiments and so cannot be a replicative precursor of stable double stranded DNA. Moreover, the base composition and lack of complementarity with ribosomal RNA indicate that this single stranded nucleolar DNA is not related to ribosomal RNA genes in the nucleolus.

The kinetics of labelling, the base composition, the nucleolar localization and the lack of complementarity with ribosomal RNA all indicate that this single stranded nucleolar DNA comprises a discrete portion of the cell genome. Its function remains unknown, but Almaric *et al.* speculate that it may act as a recognition site and have some regulatory function. The existence of single stranded stretches of duplex DNAs with this function was suggested by Crick recently.

forty years, has concluded (Medd. Grønland, **187**, 307 pp.; 1972) that the newts and salamanders (urodeles) have descended from one group of rhipidistians, and the frogs and all other tetrapods, including ourselves, from another.

His study is based on fossils from Greenland and Spitsbergen, where he has collected, notably with the English-Norwegian-Swedish Spitsbergen expedition of 1939 and thirty years later with the French expedition of 1969. Between-times he worked with the Danes in East Greenland. Much of the material described in this article was collected on these expeditions.

Jarvik usefully summarizes his life-work on rhipidistians and the origin of tetrapods. He presents new restorations of the heads of *Holoptychius*, *Glyptolepis* and *Porolepis* and gives the first full account of his serial sections through the snout region of *Glyptolepis*. There are also valuable restorations of lower jaws, gill skeletons and shoulder girdles. Jarvik's views on the dual origin of the tetrapods result from highly detailed comparisons between the heads of rhipidistians and living Amphibia. Unfortunately few other forms are known in comparable detail and, because the validity of comparative anatomy depends largely on a knowledge of as great a variety of types as possible, one would like to see similarly profound studies of other forms. Such a survey is, however, beyond the scope of an individual.



Jarvik's restoration of the skull of *Glyptolepis groenlandica* (from Medd. Grønland, **187**; 1972).

Jarvik also deals with another controversy—that of the origin of the vertebrate skull. Although it is generally accepted that the skull is segmental in organization, the idea that it is composed of modified vertebrae (first conceived by Goethe and the “natural philosophers”) has not gained many adherents. Nevertheless Jarvik provides stimulating and forceful arguments in support of this view.

In spite of the controversies, the greatest tribute is due to Professor Jarvik

and his colleagues in Stockholm who have, through patient and meticulous work carried out since the 1920s, vastly increased knowledge of extinct forms, and the status of the science of palaeontology.

PHYTOCHROME

Membrane Constituent?

from our Phytochemistry Correspondent

PHYTOCHROME is a photochromic protein present in all green plants in which it mediates a wide range of light-initiated activities ranging from rapid movements of leaflets and chloroplasts to longer term effects on metabolism and morphogenesis. Much evidence has accumulated recently that phytochrome is a membrane protein (or at least associates with membranes) and that it regulates the ionic permeability and thus the electrical potential of certain cell membranes. Roux and Yguerabide (Proc. US Nat. Acad. Sci., **70**, 762; 1973) now show that purified phytochrome can spontaneously enter and alter the conductance of artificial membranes.

Artificial bilayer membranes were constructed from 7-dehydrocholesterol and oxidized cholesterol across an aperture in a ‘Teflon’ cup and were stable for about 30 min. Phytochrome purified from oat seedlings (molecular weight about 110,000) was injected in the Pr form into the cup and the conductance was measured while the membrane was sequentially irradiated with 660 nm and 720 nm light. The red light caused a ten-fold drop in membrane conductance which, in the case of oxidized cholesterol membranes, was readily and repeatedly reversible by the far-red light. Boiled, aged or otherwise denatured phytochrome had no effects. The simplest explanation of these results is that the membrane-bound Pfr form of phytochrome induces a higher conductance than the bound Pr form.

The fact that phytochrome is a water-soluble protein with a high content of polar amino-acids has caused many plant biochemists to be reluctant to accept it as a membrane constituent, but Roux and Yguerabide point out that the molecule has an even higher content of nonpolar amino-acids. Thus phytochrome may have considerable regions of nonpolarity which are capable of interacting with the hydrophobic environment of the lipid bilayers, and possibly with intracellular membranes.

Other interesting information of the properties of phytochrome has come from the immunological experiments of Pratt (Plant Physiol., **51**, 203; 1973) who has found that purified phytochrome from peas is not immunologically identical to that from oats, rye

and barley. Phytochrome from two different cultivars of oats was identical, although that from rye and barley differed slightly from the oat samples. Cundiff and Pratt (Plant Physiol., **51**, 210; 1973) have also investigated the immunological behaviour of large and small phytochrome preparations from oats. When phytochrome is carefully isolated, it has a high molecular weight of around 240,000. Another fraction with a molecular weight of 60,000 is often found and this is produced artefactually by protease hydrolysis during extraction. Cundiff and Pratt have found that large phytochrome has at least two antigens which are not present in the small phytochrome. These two antigens cannot be detected spectrophotometrically and apparently one has a higher molecular weight than the small phytochrome fraction. Thus, proteolysis of large phytochrome yields a moiety of 60,000 molecular weight and other moieties, one larger than 60,000 molecular weight, which are not associated with the chromophore.

RNA TUMOUR VIRUS

Leukosis Agent

from our Cell Biology Correspondent

FOR some time the idea that avian leukosis viruses may in essence be deletion mutants of non-defective avian sarcoma viruses has been going the rounds without any definitive experiments being attempted. A considerable body of circumstantial evidence in support of the idea has been accumulated, but the only way to test whether a virus can cause leukosis in chicks is to inoculate birds and see how they die. There are not that many laboratories equipped for such experiments, but at Houghton Poultry Research Station, Biggs and Milne have recently tested the oncogenicity of two mutants of Schmidt Rupp Rous sarcoma virus isolated by Graf *et al.* As Graf and his colleagues had shown, these two mutant strains of Rous sarcoma virus have lost the ability to transform chick fibroblasts and they also have all the other *in vitro* properties of avian leukosis viruses. But do they have the ability to cause leukosis in chicks? According to Biggs *et al.* (J. Gen. Virol., **18**, 399; 1973) they do.

Biggs *et al.* inoculated day-old chicks from flocks of strains apparently free of leukosis and sarcoma viruses of subgroups A, B, C, and D with Graf's mutants which belong to subgroups A and D. One of the strains of chicks used is particularly susceptible to injection by sarcoma virus infection and the other is very susceptible to leukosis virus. The inoculated birds were then watched; several contracted Marek's disease and were lost from the experiment; none developed sarcomas, which

is to be expected because Graf's mutants are non-transforming. Up to 50 per cent of the birds of the strains susceptible to leukosis virus developed either erythroid or lymphoid leukosis as a result of inoculation with the non-transforming Rous sarcoma mutants. This striking result indicates either that the mutant virus genome has the capacity to induce leukosis or it is able to recombine with an endogenous, latent leukosis virus genome in the chicks. But in any event the mutant viruses can be called leukosis viruses because they induce leukosis either directly or indirectly. Furthermore these experiments establish that more than one gene in avian leukosis/sarcoma viruses can cause neoplasia.

If all chick cells did not inherit the genetic information required to specify an avian leukosis virus (using that term in its taxonomic rather than pathological sense), the interpretation of the experiments of Biggs *et al.* would be simpler and more clear cut. But the fact is that all chicks do apparently inherit a DNA provirus of a leukosis virus; where they differ is in the extent to which this endogenous viral genome is expressed. As Hayward and Hanafusa (*J. Virol.*, **11**, 157; 1973) report, chick cells which contain the group specific antigen of the avian RNA tumour viruses and the helper factor for replication of infectious BH.RSV contain between three and forty copies per cell of viral RNA which is apparently transcribed from less than about 50 per cent of the endogenous leukosis virus genome. By contrast, cells which lack the gs antigen and helper factor do not contain any detectable viral RNA but they do contain viral DNA. Further, cells which support the replication of an exogenous leukosis virus RAV-2 contain 3,000 to 4,000 copies of complete transcripts of the viral genome.

All these data are consonant with the dogma that all chick cells inherit a DNA provirus of an avian RNA "leukosis" virus and that the extent to which the endogenous genome is expressed is controlled by other cellular genes. Whether the endogenous virus has any oncogenic potential is an important question that has yet to be answered.

MAGNETIC RESONANCE

EPR in a Superconductor

from a Correspondent

THE first unequivocal observation of electron paramagnetic resonance (EPR) of a localized moment in a superconductor has been claimed by Rettori *et al.* of the University of California (*Phys. Rev. Lett.*, **30**, 437; 1973). The experiments were performed on the intermetallic compound LaRu_2 contain-

ing various concentrations of gadolinium. It is the Gd atoms which provide the local moments that are the source of the resonance signal.

The magnetic impurities reduce the tendency to superconductivity of the LaRu_2 as they act as an unpairing mechanism on the paired electrons, the so-called Cooper pairs, which are responsible for the superconductivity. For gadolinium concentrations which are not too high, however, the alloy remains a superconductor. The applied magnetic field needed to perform the resonance experiment also tends to destroy the superconductivity. But in spite of the destructive mechanisms, the alloys used by Rettori *et al.* were superconducting in the presence of the resonance field at temperatures less than about 3 K and, below 2.8 K, they were able to observe X band EPR in the superconducting state. EPR in the normal state was also observed at temperatures greater than 3.2 K.

From the results of the experiments it was possible to obtain estimates of the exchange and spin-orbit scattering rates. These rates determine how an excited Gd atom loses its energy. Initially the energy is lost to the conduction electrons by exchange scattering and then the conduction electrons lose the energy to the lattice by means of their coupling through the spin-orbit interaction. If the exchange scattering rate is higher than the spin-orbit scattering rate a "bottleneck" is formed because energy is piled up in the conduction electrons. It is rather like a tank which is being filled and emptied at the same time. If the filling rate is higher than the emptying rate then the tank becomes full and any further

filling is prevented. For the $\text{LaRu}_2\text{:Gd}$ system in the temperature range used in the experiments no bottleneck was observed in either the normal or superconducting states. But recent theory (K. Maki, unpublished) predicts an increasing exchange rate and a decreasing spin-orbit rate on going from the normal to the superconducting state. Thus, now that EPR has been observed, there is the exciting possibility that bottlenecked systems can be measured, and it should then be possible to determine directly the dynamics of the conduction electron spins in the superconducting state by making use of the changing EPR-bottleneck conditions.

GEOCHEMISTRY

Pulling Together

from a Correspondent

ENVIRONMENTAL biogeochemistry, the subject of a symposium held in Logan, Utah, from March 22 to 24, is more an area of common interest than a new discipline. The emphasis throughout the meeting was on the understanding of environmental processes involving chemistry and microbiology and the connexion between these studies and the geological record. Perhaps the principal achievement of the conference was that it highlighted the considerable current activity in the study of the interaction between microorganisms and inorganic materials. Microbiologists, chemists, soil chemists and so on often have slight knowledge of each other's disciplines, however, and it was in bringing people together to effect a cross fertilization of ideas and techniques that the confer-

Maturation of Ribosomal RNA

IN *Nature New Biology* next Wednesday (May 9) Chang and Irr report a series of experiments in which they have analysed the synthesis and maturation of ribosomal RNAs in stringent and relaxed strains of *Escherichia coli* starved for leucine. Their data indicate that in both strains ribosomal RNA genes are transcribed to yield precursor ribosomal RNAs, albeit in different amounts. Furthermore in both stringent and relaxed strains of *E. coli* in conditions of amino-acid starvation the maturation of the ribosomal precursor RNA is blocked at its terminal stages so that precursor 16S and precursor 23S ribosomal RNAs are not converted to mature 16S and mature 23S ribosomal RNAs. In other words "accumulation of rRNA precursors is not an artificial situation brought on by starvation of a mutant which has lost the ability to regulate the synthesis of RNA but, rather, a

phenomenon which occurs in both *rel⁺* and *rel⁻* bacteria during leucine starvation".

But why does amino-acid starvation, apart from any effect on RNA synthesis, block the maturation of ribosomal RNA precursors at some late stage in the maturation process? Chang and Irr suggest that certain ribosomal proteins are required to alter the configuration of precursor ribosomal RNAs and therefore protect them from complete degradation but make them available for the precise cleavages that result in correct maturation. If the pools of these ribosomal proteins are exhausted during amino-acid starvation, maturation will not go to completion. In short, Chang and Irr believe that during amino-acid starvation precursor ribosomal RNAs accumulate in *E. coli* because the supply of ribosomal proteins that are essential for maturation becomes limiting.

ence has made its most valuable contribution.

Interesting studies reported included those described by Dr T. D. Brock (University of Wisconsin) of sulphur bacteria living at 95° C and pH 2 in hot springs of Yellowstone National Park; by Dr W. E. Krumbein (Biologische Anstalt, Helgoland) of lichens which bring about the formation of "desert varnish" on the exposed rock surfaces; by Dr H. L. Ehrlich (Rensselaer Polytechnic Institute) of the micro-organisms involved in the formation and dissolution of ferromanganese oxide nodules on the ocean floors; and by Dr J. V. Beck (Brigham Young University) of those bacteria (*Thiobacillus oxidans*) responsible for accelerated leaching of copper from mine tips. Some environments were discussed at considerable length, especially those involving stromatolite formation by blue-green algae. Thus, Dr S. Golubic (Boston University) gave a fascinating account of the sequence of events taking place on the coast near Abu Dhabi in the Persian Gulf. These modern environments provide invaluable insights into the fossil record for geologists, geochemists and palaeontologists. Dr Golubic's contemporary stromatolites serve as a guide to the understanding of the Precambrian stromatolites described in talks by Dr J. W. Schopf (University of California, Los Angeles), Dr S. M. Awramik (Harvard University) and Professor P. E. Cloud (University of California, San Diego).

Other studies described by participants included investigations of aquatic environments in the Everglades of Florida, the Severn Estuary and certain lakes in the British Lake District and North America, and the Arctic Seas. Mathematical models built up in small computers are now in common use by geochemists and ecologists for the study of element, energy and mass balances within individual organisms and complete ecosystems. The kinetic models of Dr R. A. Berner (Yale University) for the early diagenesis of nitrogen, phosphorus and sulphur in anoxic marine sediments were described by Dr I. Kaplan (University of California, Los Angeles) and other speakers as helpful though incomplete treatments of very complex situations. In this context it is clear that pollution studies have to be seen in relation to the natural fate and the natural variability of elements and compounds in environments. This was illustrated by the work of the group at the University of Bristol (described by Dr G. Eglinton) on the short-term fate of selected biolipids (oleic acid, phytol and cholesterol) and of DDT in estuarine and lacustrine sediments.

The meeting concluded with a field trip to the algal mats at the north

shore of the Great Salt Lake. Such a trip should be an integral part of any future conference, for it provides a focus for first-hand joint experience and discussion of a particular ecosystem. The interfaces between biosphere and geosphere need just this type of interdisciplinary attack. There was enthusiastic support for the organization of another conference in two years time with participation extended to include natural product chemists, biochemists, kineticists and mathematicians so that the problems can be more thoroughly discussed.

MICROWAVES

Expanding above 10 GHz

from a Correspondent

A FEATURE of radio research and development in recent years has been the increasing interest in propagation at frequencies greater than about 10 GHz. The large bandwidth available at these frequencies is particularly attractive in terrestrial and space communication systems for additional channels of information. Absorption and scattering in the lower atmosphere also becomes more important, however, as the frequency increases about 10 GHz. Consequently, practical exploitation of this part of the spectrum requires extensive basic research on wave propagation. This dual theme of the development of communication systems and the limitations imposed by meteorological factors was

emphasized at the international conference held between April 10 and 13 at the Institution of Electrical Engineers.

In his opening address Mr J. H. H. Merriman, deputy president of the IEE, pointed out that the upper limit of radiofrequencies discussed within the context of international control was increasing by a factor of about ten every decade. Frequencies up to nearly 300 GHz (wavelengths down to 1 mm) have provisionally been allocated for various requirements. A topic of particular interest is the reliability of terrestrial radio relay systems above 10 GHz, and the prediction of their performance from radio meteorological data. Very heavy rain can cause serious fading and this effect increases steadily with increasing frequency. Contributions from authors at the Radio and Space Research Station of the Science Research Council and from the Post Office Research Department described a comprehensive experiment to investigate this problem. Simultaneous measurements of rainfall structure and of fading on several links at frequencies of 11, 20 and 37 GHz are in progress in Suffolk. From this work it is hoped to predict the optimum lengths and spacings of microwave links to avoid the worst effects of heavy rain. Members of the Post Office Research Department also discussed the fading ("multipath propagation") which can occasionally occur in clear conditions on terrestrial links as a result of abnormal refraction. These multipath effects are particularly important in view

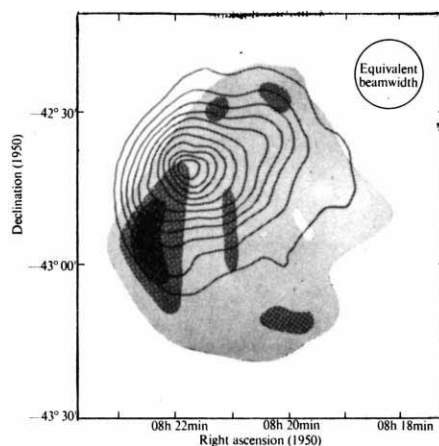
Puppis A Mapped by Copernicus

THE supernova remnant Puppis A has been mapped in the energy range 0.5 to 1.5 keV with better resolution than in any previous observations. The X-ray emission is found by Zarnecki and his colleagues to be strongly peaked and concentrated chiefly in the north-east quadrant of the radio shell (see next Monday's *Nature Physical Science*, May 7).

Previously, one-dimensional scans of Puppis A and rocket borne collimated proportional counters have shown that the angular size of the X-ray emitting region is less than 0.5 arc degree. The source is one of the brightest known at X-ray frequencies less than 2 keV and the X-ray spectrum is best fitted by a combination of thermal bremsstrahlung and line emission from a plasma at 4×10^6 K. The latest map, however, provides the first data obtained with high spatial resolution in two dimensions.

The data are not consistent with a simple point source and disk model of the source, and the X-ray emission is contained within the corresponding

radio shell. A compact object may remain inside the remnant, but the evidence is not conclusive in this respect. On the whole, Zarnecki *et al.* feel that the observed X-ray and radio features can be adequately explained by existing supernova models.



Contour map of Puppis A at soft X-ray frequencies (shaded). Solid contours are radio observations, after Milne (*Austral. J. Phys.*, 24, 429; 1971).

of the wide bandwidths now required for various kinds of data transmission.

Mr R. W. Wilson and Mrs W. L. Mammel (Bell Telephone Laboratories) and Professor D. B. Hodge (Ohio State University) presented data from spaced-receiver measurements of Earth-space attenuation. At a frequency of 16 GHz, receiver separations of 10–15 km in a switched-path network give a significant improvement in reliability for the locations used in the United States. As the results presented by Dr P. G. Davies (Radio and Space Research Station) showed, however, there is a considerable variability from year to year at a given location and also from one climate to another. Results from RSRS illustrated that South-east England has an excellent climate from the point of view of reliable space communication. Even frequencies as high as 100 GHz may be useful there for short-hop terrestrial paths or Earth-space links in or near the vertical.

Several contributors presented results of radiometric measurements of thermal emission noise from the troposphere. This technique affords a simple method of deriving attenuation values of up to 10–12 dB on Earth-space links. Radiometers have also been used to study thermal noise from the local environment around the site of an Earth station. The results of Mr F. V. C. Mendis and Dr T. Pratt (University of Birmingham) and of Mr D. M. Knox, Mr R. G. Howell and Mr J. A. McLeod (Post Office), at 9 and 17 GHz respectively, gave values for the noise radiated from buildings, foliage, and various types of ground surface. In general, however, the contribution from the sky noise is more important than that from local ground-based sources.

For reasons of economy of space in the microwave spectrum, sharing of frequencies by transmissions using orthogonal polarizations has been proposed. Contributions from the universities of Bradford and Essex and from the Post Office dealt with theoretical and experimental work on this topic. Although it seems likely that distortion of the plane of polarization by the constituents of the troposphere will not prove serious on short links at frequencies of about 20 GHz, further work is required at a range of frequencies and in various weather conditions. Studies of the shape and orientation of raindrops are also necessary to extend the theory of cross-polarization. This is one example of a general requirement for basic radio meteorological data to assist future developments in communications. In spite of much important work related to forecasting requirements in meteorology, remarkably little is known about the varying structure of heavy rain over distances important in microwave communication, of the order of a kilometre.

Several contributors demonstrated that, quite apart from communication

interests, frequencies greater than 10 GHz have special advantages in remote probing of tropospheric structure. For example, anomalous results, difficult to explain by existing theories, were shown in measurements of atmospheric emission at 15 GHz by Dr M. S. Reid and Mr T. J. Lockhart (California Institute of Technology and Air Associates). Similarly, at 85–118 GHz, the results of Dr C. J. Gibbins, Mr A. C. Gordon-Smith and Dr D. L. Croom (Radio and Space Research Station) showed a greater variability in emission noise than expected on the basis of data on the water vapour content and temperature structure. There are probably features of atmospheric structure (such as molecular complexes and the effect of minor constituents) which are not yet understood. Frequencies greater than 10 GHz will, in this respect, prove a happy hunting ground for radio physicists.

Time of the Corsica-Sardinia Rotation

THERE is now convincing evidence that Corsica and Sardinia have rotated in an anticlockwise direction from positions adjacent to southern France. Such a rotation is consistent with palaeomagnetic data from Corsican Permo-Carboniferous rocks and from Permian and Oligo-Miocene rocks of Sardinia. Moreover, Corsica makes a good fit with France along the 1,000 m isobath, enabling Permo-Carboniferous outcrops in Corsica to be matched with similar outcrops in Provence and permitting the alignment of offshore magnetic anomalies thought to result from andesitic volcanoes.

But there is perhaps less agreement on precisely when the rotation took place, estimates of the time of rotation having ranged from Triassic to Miocene. Some years ago, Nairn and Westphal (*Palaeogeog.*, *Palaeoclimatol.*, *Palaeoecol.*, **5**, 179; 1968), for example, suggested that the rotation may have occurred during the Mesozoic, although more recently, and in the light of additional data, Alvarez (*Nature Physical Science*, **235**, 103; 1972) proposed a Tertiary rotation. More specifically, Alvarez adduced evidence showing that Corsica-Sardinia could not have separated from France before 11 or 12 million years ago. The completion of the rotation, on the other hand, was less easily timed, although the presence under the Ligurian Sea floor of salt domes derived from upper Miocene evaporites led Alvarez to conclude that rotation must have ended early enough to allow the evaporite deposition during part of the Messinian period, 7–5 million years ago.

In next Monday's *Nature Physical Science* (May 7), Alvarez reports a further attempt to determine the time of completion of the rotation, based on

NUCLEAR PHYSICS

Recoil and Transfer

from a Correspondent

VERY many investigations have established the one-nucleon transfer reaction as a powerful way of investigating the single-particle character of nuclear states. Most of this work has used incident particles consisting of rather few nucleons, in particular the familiar (d,p) and (d,n) reactions, and also reactions initiated by tritons, helions and alpha particles.

In recent years, however, heavy ions have come into their own now that they can be accelerated to energies sufficiently high to surmount the Coulomb barrier of the nucleus and interact with the nuclear field. These reactions have several features that make it useful to study their properties in detail. In par-

the palaeomagnetism of Plio-Pleistocene basalts from north-west Sardinia. Two distinct generations of basalt (designated β_1 and β_2) were represented in the collection examined. Unfortunately, because of dating problems, absolute ages are available for neither set; but the younger generation (β_2) are associated with cinder cones almost unaffected by erosion and must therefore be late Pleistocene or Holocene, and the older set (β_1) are regarded as Pliocene or possibly late Miocene.

The mean palaeomagnetic direction from the β_2 samples (declination 350° , inclination $+51^\circ$) agrees closely with that (353° , $+53^\circ$) recently obtained by Bobier and Coulon (*CR Acad. Sci. Paris*, **270**, 1434; 1970). Both directions, and all other mean palaeomagnetic directions from Sardinian basalts which are definitely post-Miocene, cluster around the present field direction, showing that the rotation must have been completed before the end of the late Miocene (about 10 million years ago). Because samples from one of the three (older) β_1 sites turned out to have an intermediate direction, Alvarez calculated no β_1 mean palaeomagnetic direction from his data alone. The overall mean (354° , $+56^\circ$) calculated from Alvarez's and other sources of β_1 data is, however, very close to that (352° , $+48^\circ$) obtained from all β_2 data, showing that, taken at face value and neglecting errors, the (older) β_1 mean is actually closer to the present field direction (0° , $+60^\circ$) than is the (younger) β_2 mean. Thus, as Alvarez points out, the β_1 data do not confirm the suggestion from Manzoni *et al.* (*Giornale di Geologia*, **38**, 5; 1972) that the β_1 basalts predate the completion of rotation—both generations of basalt seem to postdate it.

ticular, they often show semi-classical characteristics that simplify the analysis of the cross-sections, and also the heavy ions bring in high angular momenta that make it possible to excite states of high spin. Furthermore, these reactions often show marked selectivity in that they tend preferentially to populate states of a particularly simple structure.

The distorted wave Born approximation (DWBA) formalism has been used with great success to analyse the (d,p) and similar reactions; it has yielded important information on nuclear structure, particularly the spectroscopic factors. This theory has naturally also been applied to one-nucleon transfer reactions between heavy ions and it has been found to give excellent results in some cases but not in others.

A particularly notable discrepancy is found for reactions between light nuclei. These have cross-sections that fall off steadily with increasing angle, whereas the DWBA predicts angular distributions showing a marked oscillatory structure. The reason for this is that in conventional DWBA calculations the nuclear recoil is neglected, and this is justified if the mass of the incident particle is much less than that of the target nucleus. In the case of reactions between heavy ions these masses are comparable and recoil can no longer be ignored.

Examination of the angular momentum conservation relations in the reactions shows that if recoil is neglected

the change Δl in the orbital angular momentum of the transferred particle must be zero, whereas if recoil is taken into account Δl may be 0 or 1.

The inclusion of recoil effects makes the calculations much more complicated, but it still remains within the capacity of fast computers. A program including recoil effects has recently been written and DeVries and Kubo (*Phys. Rev. Lett.*, **30**, 325; 1973) have used it to investigate the importance of these effects. They find, as shown in the diagram, that the $\Delta l=0$ and $\Delta l=1$ contributions to the reaction amplitude both oscillate as a function of reaction angle, but that the oscillations are out of phase so that their sum varies smoothly with angle, just like the experimental data.

This shows very clearly that recoil effects must be included in DWBA calculations of nucleon transfer between heavy ions of comparable mass.

The calculations also give the product of the two spectroscopic factors for the transition, and this is close to the theoretical value of Cohen and Kurath. Thus the DWBA theory is able to give a good account of both the angular distribution and the absolute magnitude of the cross-section for single-nucleon transfer between heavy ions. It now seems likely, subject to further tests, that these reactions can be used with some confidence to determine unknown spectroscopic factors and will thus become an important tool for the determination of nuclear structure.

Ultrasonic Velocities in Troodos (Cyprus) Rocks

ALTHOUGH the idea that the Troodos Igneous Complex of Cyprus is an upthrust segment of (Mesozoic) seafloor has been around now for about a decade, the evidence to settle the matter with certainty has still not been obtained. Nevertheless, most, if not all, of the relevant geophysical data currently available are consistent with the seafloor interpretation, suggesting that any refutation of the idea in the future is unlikely.

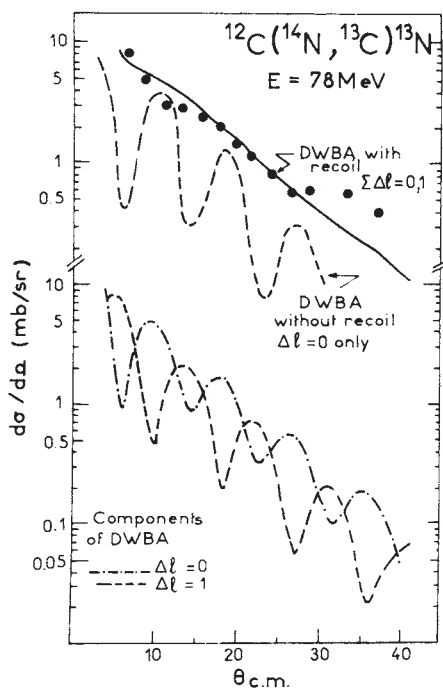
In the meantime, the search for that vital piece of evidence goes on, largely by geophysical means. One of the obvious approaches to the problem is the measurement of seismic velocities, the hope being that the velocity structure of the Troodos Massif will prove to correspond with the unique structure of the present ocean floor rather than with the variable structures of continents. Matthews *et al.* (*Nature Physical Science*, **231**, 200; 1971), Lort and Matthews (*Geophys. J.*, **27**, 383; 1972) and Khan *et al.* (*Nature Physical Science*, **238**, 134; 1972) have all made seismic determinations in the field; but through no fault of these workers, all these investigations have been less than ideal. One of the principal difficulties is that, being upthrust, the Troodos Complex is no longer subject to the confining pressures typical of present or ancient ocean floor *in situ*, so that seismic velocities can hardly be expected to correspond directly. In addition, fracturing in the Troodos units has almost certainly reduced seismic velocities from those applicable at ocean floor depths.

In next Monday's *Nature Physical Science* (May 7), Poster reports attempts to solve these problems by measuring velocities in Troodos samples under pressure in the laboratory. He has determined ultrasonic velocities under hydrostatic pressures of up to 2 kbar and at room temperature for nineteen

rock samples representative of each of the principal Troodos units. Typically, velocities increase rapidly up to pressures of about 1 kbar and thereafter remain relatively constant, the initial increase being most marked for the pillow lavas and rocks from the sheeted intrusive complex. But even at atmospheric pressure, velocities were higher than those measured in the field, presumably because of the relative lack of fracturing in the smaller laboratory samples. This velocity difference was greatest in rocks of the plutonic complex (gabbro and ultramafics).

With the exception of the ultramafics, the velocities measured in the laboratory increased with downward stratigraphic order, encouraging Poster to compare the velocity structure with that of "normal" oceanic crust (assuming a purely confining pressure to be exerted on the top of layer 2). At the relevant pressures of 0.5–1.0 kbar, the pillow lava velocities were lower than, and the velocities in the sheeted intrusive complex were generally higher than, the average for oceanic layer 2 (5.1 km s⁻¹). Thus the basal group of the sheeted intrusive complex, comprising screens of pillow lavas with diabase dykes and thought to correspond with layer 2, could easily produce the mean velocity of layer 2 and the wide range of velocities observed in it. Stratigraphically below, measured velocities then increased progressively to typical layer 3 velocities for the fresh gabbros.

The new velocity data thus generally support the view that the Troodos Complex began as oceanic crust, the only anomaly being the fact that the ultramafics have lower velocities at all pressures than the gabbros which lie stratigraphically above. Poster's explanation for this discrepancy is based on his discovery that seven of the ten ultramafics examined were at least 75 per cent serpentized.



Differential cross-section for a one-nucleon heavy ion transfer reaction compared with DWBA calculations. The upper dashed curve shows the DWBA calculations without recoil ($\Delta l=0$ only) and the two lower curves the DWBA calculations with recoil ($\Delta l=0$ and 1). The sum of these two lower curves is the full curve, which agree well with the experimental data.

Experiments on Polishing of Diamond

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Polished diamond surfaces have unusual properties because polishing of the brittle material generally proceeds by mechanical chipping.

DIAMONDS are shaped and polished to produce gem stones by methods which have remained essentially unchanged for several hundred years. Yet it is only recently that the physical basis of these operations has been studied, and their unusual features fully appreciated. This article outlines the principal features of the polishing process and describes some recent experiments which illustrate the very characteristic nature both of the process itself and the polished surfaces produced. We shall show that these perhaps unique properties result from the very hard and brittle nature of the diamond.

The usual method of polishing a diamond is to hold it either against the face of a rotating cast-iron wheel (or scaife) charged with a suspension of fine diamond powder in a light oil, or against the face of a wheel in which diamond powder is bonded into a metal matrix. One of the most striking features of this method is that if a cube face is prepared on the diamond, it turns out that the rate of removal of material during polishing with a bonded wheel in a direction parallel to a cube axis is about 100 times greater than if the same face is polished in a direction at 45° to a cube axis. Similar effects are observed on other faces, and even greater differences are evident when polishing on a cast-iron scaife, as the more resistant directions of the diamond tend to knock the powder out of the scaife.

In preparing a gem stone from a rough diamond it is necessary to remove a certain amount of material to position the facets before polishing them. Another unusual feature is that this removal of material is effected in almost the same way as polishing, except that larger sized diamond powder is used to remove material quickly. For most materials, polishing and the removal of material by abrasion are quite different processes. Removal of material is generally achieved by the use of an abrasive powder in what is essentially a cutting process; the powder is harder than the specimen, and it either gouges out material, or sets up stresses which lead to cracking after it has moved on. By contrast, polishing is generally a smearing process, brought about by rubbing with a second material to produce high temperatures in the small regions of true contact between the asperities on the two surfaces; the high temperatures result in local melting of the asperities, which are then smoothed out. It follows that to polish a specimen, we must use polishing material with a higher melting point, so that on rubbing, the specimen melts and flows first. For further details of the general features of abrasion and polishing, see for example, Bowden and Tabor¹ and Cottrell².

In view of all this, one would expect to encounter difficulties both in removing material from diamonds and in polishing them. As far as the removal of material is concerned, there is no harder substance than diamond which can be used to produce abrasive cutting. In rubbing operations, the high thermal conductivity of diamond will tend to

reduce the temperature of the hot spots at the areas of local contact, whereas high temperatures are required to effect even the conversion of diamond to graphite. Thus, conditions seem unfavourable for both abrasion and polishing, but in fact the traditional methods used by the diamond industry abrade and polish diamond quite successfully. The rate of abrasion is relatively low, but a high polish can readily be obtained, and a diamond surface may be worked to the standard of a good optical flat³.

The first scientific experiments on the polishing process were made more than 50 years ago by Tolkowsky⁴, who proposed that both abrasion and polishing proceed by mechanical chipping on a microscopic scale. This chipping will be controlled by the lie of the cleavage planes relative to the direction of abrasion, and will therefore occur more readily in some directions than others, thus giving rise to very considerable differences in rates of abrasion. To indicate the geometrical arrangement of the cleavage planes, Tolkowsky made use of a model of diamond built up from small identical octahedral and tetrahedral shaped blocks, and thus explained the variations of the hardness on both the cube and the dodecahedron planes^{4,5}.

Tolkowsky was principally interested in practical applications and his conclusions about how to polish and abrade diamonds soon found their way into the diamond trade, as did his other research on the best way to lay out the facets of a diamond so as to produce the well known brilliant cut. On the other hand, his account of the underlying mechanism was not widely published, and attracted little attention. His treatment was criticized on the grounds that diamond is certainly not built up of equally sized elementary blocks as in his model, but the essential function of the model is to indicate the positions of the cleavage planes—and this it does correctly. It now turns out, as the result of various experiments on diamond over the past 15 years, together with increasing knowledge of the nature of brittle materials, that Tolkowsky's views are essentially correct.

One of the crucial features of the above experiment observed but not stressed by Tolkowsky⁴, is that the rate of removal of material is proportional to the total number of revolutions of the polishing scaife but does not depend on its speed. This result, confirmed by Wilks and Wilks^{5,6}, shows that thermally activated processes play no significant part in the removal of material. The rubbing together of two materials produces heating at the areas of true local contact, and this excess temperature increases rapidly with the speed of rubbing¹. The fact that the abrasion per revolution does not increase with rising speed of rubbing clearly demonstrates that local hot spots play no significant part in the wear process. In particular, it rules out the possibility that the diamond is worn away by either burning or conversion to graphite, as suggested by Seal⁷. On the other hand, one would expect the same amount of material to be removed by each revolution of the wheel if the abrasion proceeds by a mechanical chipping or cleavage process.

The conditions necessary for materials to fail by chipping or brittle fracture have been discussed by several authors, for example Kelly⁸. Kelly, Tyson and Cottrell⁹ have considered whether an ideal crystal free of imperfections will fail plastically by shear or by brittle fracture under tension. They show that for face centred metals such as copper,

gold and silver, the stress required to produce fracture is about 30 times greater than that to produce shear, and that failure always occurs by plastic deformation rather than cleavage. On the other hand, the calculations show that, of the substances considered, sodium chloride and diamond are most likely to fail by brittle fracture. The critical stresses for shear and cleavage in sodium chloride and diamond are approximately equal, but the calculations are not sufficiently refined to indicate which process is preferred. These calculations are, however, for ideal crystals and the essential question for real materials is whether the presence and motion of dislocations will permit plastic flow before fracture.

Dislocations in diamond have been observed by Evans¹⁰ who prepared specimens of diamond by etching, and viewed the dislocations directly in the transmission electron microscope. They are also clearly delineated by the X-ray topograph techniques of Frank and Lang¹¹. Calculations mentioned by Evans¹² suggest that the movement of the dislocations will be inhibited by the necessity of breaking carbon-carbon bonds. Their motion has been studied experimentally by Evans and Wild^{12,13} who took specimens of diamond in the form of thin slabs, loaded them in the middle, and observed whether they failed by fracture or plastic deformation. They found that at low temperatures the beam always failed by fracture, but that an increasing amount of plastic flow was observed as the temperature exceeded 1,500°C. Hence dislocations in diamond may be quite mobile, but at room temperature are held back by obstacles which can only be overcome by considerable thermal activation. As the rates of abrasion and polish in the experiments referred to above were independent of the temperature of local hot spots, one concludes that the abrasion was not accompanied by any thermally activated motion of dislocations.

It is sometimes suggested that plastic flow in diamond may be produced by conditions of intense local pressure. The effect of pressure on brittle materials is sometimes surprising, for example it is possible to compress a slab of brittle material, such as rock salt, to half its initial thickness without producing cracking¹⁴, and Howes and Tolansky¹⁵ have argued that the ring cracks produced by indenting diamond with steel spheres show evidence of plastic flow. Later studies by Lawn and Komatsu¹⁶ of the region round such a crack, using X-ray topography, show no sign of plastic deformation, and Frank and Lawn¹⁷ have since shown that the cracks may be described in terms of fracture processes.

We conclude that the mechanism of abrasion is one of mechanical chipping. This conclusion is supported by a study of the area around an abrasion mark, by Frank, Lawn, Lang

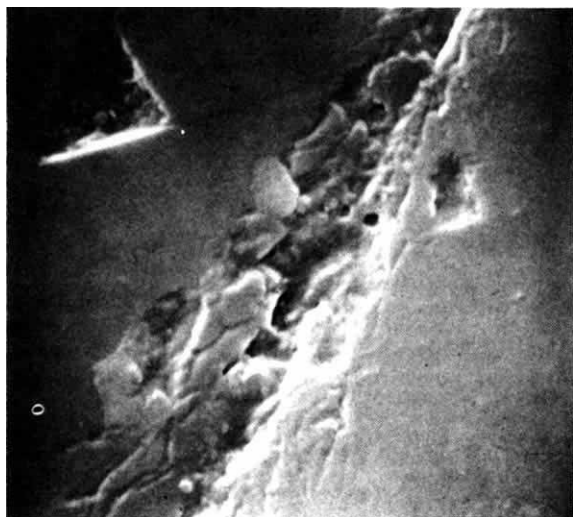


Fig. 1 Scanning electron microscope micrograph of a diamond surface indented with a Knoop indenter ($\times 8,400$).

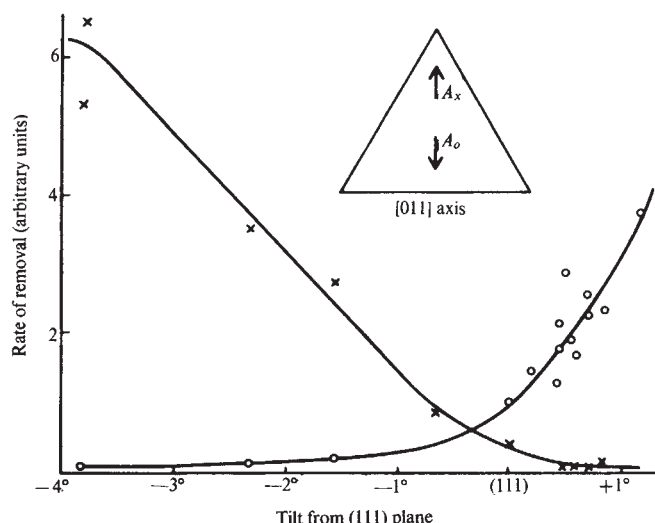


Fig. 2 The relative rates of removal of material by grinding on surfaces near to an octahedron plane. The symbols $\circ$ and $\times$ correspond to the directions shown in the inset.

and Wilks¹⁸ using X-ray topography, which shows that the state of strain in the surface is consistent with stresses set up by fracture processes and not by plastic flow. We believe that there is no convincing evidence for the motion of dislocations in any of the relevant abrasion and polishing experiments, although claims to have observed plastic flow in diamond at room temperature have been made by Brookes¹⁹ and by Gane and Cox²⁰. Brooks indented a diamond surface with a Knoop indenter made of diamond and claimed that the form of the indentation indicated that plastic flow had occurred. A careful study of Brookes's indentations, however, show clear indications of brittle fracture (Fig. 1), which appear to have been obscured in the earlier micrographs. The observations of Gane and Cox were based on the study of thin diamond wedges pressed against each other, but their evidence is at best inconclusive. Even under these rather specialized conditions, there is no clear evidence that plastic flow and motion of dislocations took place.

We shall now describe three sets of experiments which confirm the fracture process and illustrates the unusual properties of polished diamond surfaces. In the first set a simple micro-abrasion tester²¹ was used to study the variation of the rate of removal of material—in short the hardness of the diamond—with the orientation of the surface and the direction of polish. The hardness is often extremely sensitive to any change in the orientation of the facet being abraded. For example, Fig. 2 shows the relative rates of removal of material on surfaces obtained by tilting an octahedron plane about the $[011]$ axis indicated in the sketch plan. The crosses show the rate of removal of material for abrasion in the direction A_x , and the circles the rate in the direction A_o . We see that on a true octahedron plane the rate of removal in the direction A_o is more than twice that in the direction A_x , but that a tilt of only about 1° is sufficient to reverse the relative hardness.

These orientation effects are not explained by Tolokowsky's treatment, which also has difficulty in discussing the octahedron face. The variation in rates of abrasion on cube and dodecahedron faces is explained by the lie of the cleavage planes which results in the production of a different structure by abrasion in different directions. As the cleavage planes run parallel to an octahedron face, the treatment seems to predict a uniform surface with no easy and hard directions of abrasion, in contrast with the measurements. To explain this point, we must consider the behaviour of tilted octahedron facets.

A diamond surface which has been polished by a process of mechanical chipping will not be automatically flat but must consist of hills and valleys bounded by an irregular arrangement of cleavage planes as shown very schematically in Fig. 3A. It follows that abrasion proceeds by the removal of material from the tops or sides of the microhills. If Fig. 3A represents a section through an octahedron face, parallel to the directions A_x and A_o , the lie of the cleavage planes is such that the most likely sites for the removal of material are the edges a and b (ref. 5). It also follows that the easiest direction of abrasion is A_o , and that most of the material is then removed from the edges b . If, on the other hand, a face is prepared inclined to the octahedron face by a small angle, the lie of the cleavage planes remains the same, so the structure of the microhills is as shown schematically in Fig. 3B. Topographically, the abrading wheel must be presented with an increased number of b type edges, and the hardness correspondingly reduced. Similarly a tilt in the opposite direction increases the hardness.

One also expects that the change in hardness with tilt may be very rapid. If the linear dimensions of the microhills are all of the same order of magnitude h , the average distance between hills in contact with the abrading wheel will be much larger because the areas of real contact are less than the nominal areas by factors of order 10^4 . Thus the distance between the hills may be of the order of $100h$, so that a tilt of 1° would be sufficient to change the topography of Fig. 3A to that of Fig. 3B, and thus approximately double the number of sites of easy abrasion. Similar arguments may be applied to account for the hardness of both true and tilted cube and dodecahedron faces. All the predictions that can be made from the model are in accord with experiment⁵.

We have also made studies of the jagged nature of polished diamond surfaces, implied by our abrasion experiments, by observing the friction of diamond sliding on diamond in air. Some time ago Seal⁷ showed that the friction of a polished cube surface varies with the azimuthal angle of sliding, with a four-fold symmetry. This result is at first surprising, as Bowden and Hanwell²² have shown that diamond surfaces in air are covered by a strongly adsorbed film which reduces the friction by an order of magnitude below its value for clean surfaces in high vacuum. Thus even though the diamond surface is covered by a tenacious film of adsorbed gases, the symmetry of the diamond is projected through the film.

The friction between diamonds in air was first measured by Bowden and Young²³, and Bowden and Tabor²⁴ later pointed out that their results showed an approximate relation between the friction μ and the load W of the form $\mu \propto W^{-1/3}$. They then suggested that this relation arose because of adhesion processes between the actual areas of true contact, and that this adhesion was responsible for the friction. Recent measurements by Casey and Wilks²⁵ show, however, that the friction is independent of the load over a wide range of loads, including the range of the earlier experiments. It therefore seems that the mechanism responsible for friction is not one of adhesion, a result which is confirmed by the fact that the friction remains unchanged when the diamond surface is lubricated with light oil.

The observed behaviour of the friction is readily explained by a roughness (or ratchet) type mechanism associated with the jagged nature of a polished diamond surface. That is, the friction force arises from the work required to move the two diamond surfaces against the normal load as they are forced apart when asperities ride over each other. Some of this work will degenerate into heat when the jagged and irregular surfaces come together again, because the return motion will tend to be abrupt and irreversible. The work of separation is proportional to the load, so this type of mechanism leads to a value of the friction which is independent of the load. We believe that this mechanism accounts

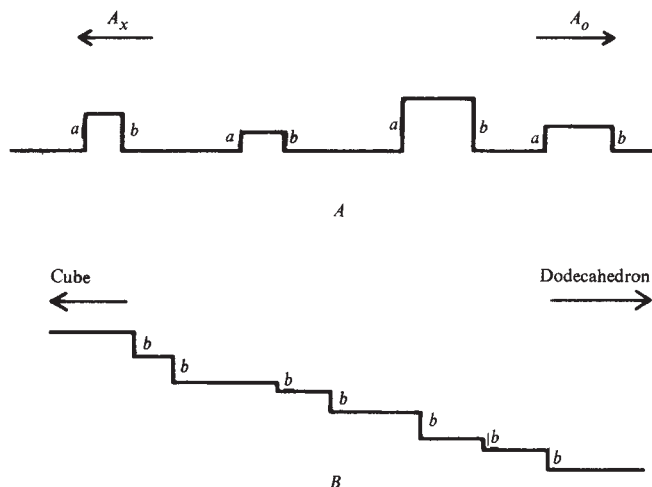


Fig. 3 Schematic diagrams of polished diamond surfaces (see text).

quite generally for the friction of diamond on diamond in air as discussed in more detail by Casey and Wilks²⁵. The adsorbed film acts as a lubricant, which reduces adhesion and the high friction observed in a vacuum, but does not obscure the structure of the surface.

Fig. 4a shows the results of recent measurements on the friction of a diamond sliding over a polished cube surface which are similar to those of Seal⁷. The diamond surface had been polished in the usual way and was therefore covered by an array of fine polishing lines or grooves running parallel to a cube axis. Nevertheless, the friction shows a full four-fold symmetry, having the same value in directions parallel and perpendicular to the grooves. We then repolished a surface by abrasion in a hard direction at 45° to a cube axis, until the original polishing lines were replaced by a new set at 45° to the original direction. The friction on this new surface is shown in curve b ; the friction has quite different values and exhibits only a two-fold symmetry, the lowest values being for directions parallel to the direction of polish.

These results confirm that the topography of a polished surface is determined by mechanical chipping. Just as abrasion proceeds at different rates in different directions, because of the orientation of the cleavage planes, so surfaces prepared by polishing in different directions will have

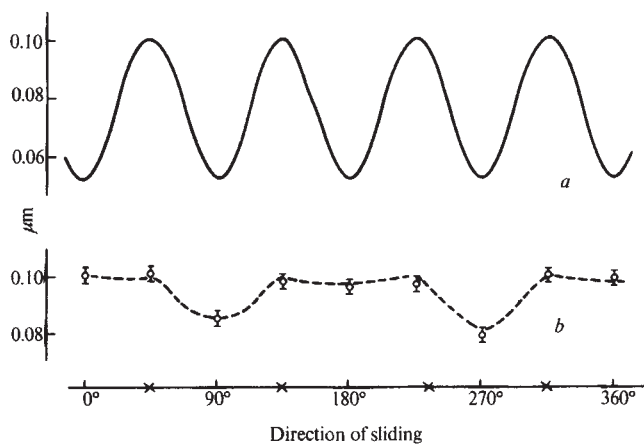


Fig. 4 The friction of a diamond sliding over a polished cube surface as a function of azimuthal angle. a , After polishing in the normal soft direction; b , after polishing in a hard direction. The symbols $\times$ indicate the positions of the cube axes.

different structures. As discussed by Tolkowsky, a surface polished in the usual direction parallel to a cube axis will present a jagged surface with a four-fold symmetry. If, however, the diamond is polished in one of the hard directions, the surface structure will tend to take the form of grooving parallel to the direction of polish, with the two-fold symmetry shown in the friction measurements. We are at present extending our studies to observe the wear effects associated with repeated passes of the stylus over the same area of surface.

The third set of experiments are concerned with observing the surface structure of polished diamonds by using high resolution electron microscopy and carbon replica techniques. The structure turns out to be irregular and on a very small scale; therefore, particular care must be taken to ensure that the micrograph is in focus and free of astigmatism. Fig. 5 shows micrographs prepared under carefully controlled and similar conditions, except that the replica of Fig. 5a was taken from a freshly cleaved surface of mica, and those of Fig. 5b and c from polished surfaces of diamond. The greater contrast in the diamond micrographs indicates that the surface is much rougher than the mica and that the scale of the irregularities is of the order of 50 Å.

The micrographs in Fig. 5b and c were prepared from the same stone, using the same technique, but Fig. 5b was taken from a cube surface polished in the usual way, and

Fig. 5c from a polished octahedron face. We have also observed similar structures on the cube and octahedron faces of a second diamond, and also a different characteristic structure on the dodecahedron faces. These results confirm the fact, well known to diamond polishers, that the quality of polish depends on the particular face being polished, as follows from the mechanical nature of the polishing process. One expects the surface structure to be delineated, at least to some extent, by cleavage planes. On a cube surface, the traces of these cleavage planes will lie at 45° to the direction of polish, and it is interesting to note that there are features lying in these directions. Further details of our results, together with details of the replication technique, will be published elsewhere.

Diamond is perhaps unique in that an apparently highly polished surface may in fact be mechanically rough on a microscopic scale, a result which is the consequence of its brittleness. We are at present applying these results to the use of diamond as cutting tools for machining metals, as both the rate of wear of the diamond, and the finish produced on the metal, depend sensitively on the surface structure of the tool. For example, the rate of wear of two single-diamond turning tools with identical external geometry, but fabricated so that the diamonds have different crystallographic orientations, show wear rates which are reproducible, and differ by a factor of 7 (ref. 26). The wear in this particular experiment, in which the diamonds turned an aluminium-silicon alloy of the type used to manufacture motor car pistons, was almost certainly controlled by the lie of the fracture planes as discussed above, and these are quite different in the two crystallographic orientations. Further experiments are being continued on both the wear of diamonds and the finish they produce.

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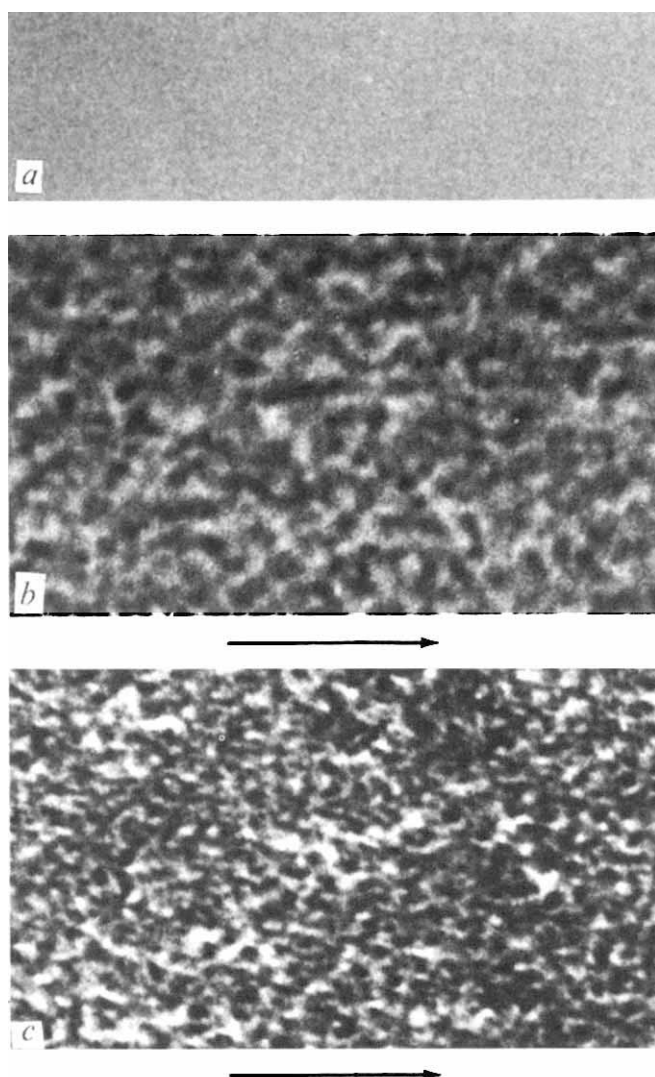


Fig. 5 Micrographs of replicas taken from a, cleaved mica; b, a polished cube face of diamond; c, a polished octahedron face of diamond. The arrows indicate the directions of polish. ($\times 1,000,000$).

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Tectono-eustatic Changes in Sea Level and Seafloor Spreading

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This article describes a model for estimating eustatic changes and evidence for a correlation with seafloor spreading.

EUSTATIC changes in sea level are caused by a change in the form of the Earth's crust or in the total volume of ocean water¹. The best examples of the latter are the short duration ($\sim 10^5$ yr) reversible changes caused by glacial advance and retreat during the Quaternary although there may also have been an increase in water volume by degassing from the mantle during the Mesozoic and Cainozoic^{2,3}. Changes in sea level arising from changes in the form of the ocean basins are revealed in the geological record on continents as longer period ($\sim 10^7$ yr) transgressions and regressions that mark global changes in sea level^{4,5}, although the magnitude of the change may vary locally. Five principal factors^{1,3,6} control sea level; of these, sediment deposition and trench formation may be neglected because the volumes are small or balanced by subduction. The most significant factors are (i) subsidence of the ocean crust, (ii) uplift of mid-ocean ridges and (iii) an increase or decrease in the length of the mid-ocean ridge system. These factors are, of course, controlled by seafloor spreading and their relation to eustatic changes has been subject to speculation⁵⁻¹³.

Simple Quantitative Model

Here we present a model designed to estimate the magnitude of the eustatic change in sea level that would result from net alterations in the distribution of matter forming the continents and ocean floor, irrespective of the mechanisms involved. We assume a constant volume of water in the oceans and that there is no significant change in the average radius, shape or rotation of the Earth. On a smooth almost spherical Earth covered with the present volume of water, the ocean floor would be about 2,440 m below sea level, which would itself be about 240 m above present sea level^{14,15}.

Interaction between tectonics and sea level begins only when part of the lithosphere is raised above sea level; then any net vertical movement between the ocean floor and dry area will obviously produce a change in sea level. In particular, any increase in volume of solid crust above the water line must also be accompanied by an absolute lowering of sea level. In the context of plate tectonics, it is most meaningful to retain the distinction between continents and oceans in calculating departures from present continental areas and ocean depths. The global hypsographic curve^{16,17} provides a convenient means of analysis (Fig. 1a) because constancy of water volume and rock density require that the total area representing crust and water volume must remain constant. To study a systematic series of deviations from the present curve, we varied the continent area (x) and maximum ocean depth (y) independently in 5% increments on either side of the present values, thereby defining forty-eight additional hypsographic curves. The base line for definition of the hypothetical continental areas was taken as the present area of continents at the altitude of present sea level, thus retaining

the geophysical concept of a continent which may or may not be covered by water. Further, the profile of the continents was assumed to remain similar to the present profile and the oceanic part was altered by an amount proportional to its depth at all points, thereby preserving important features

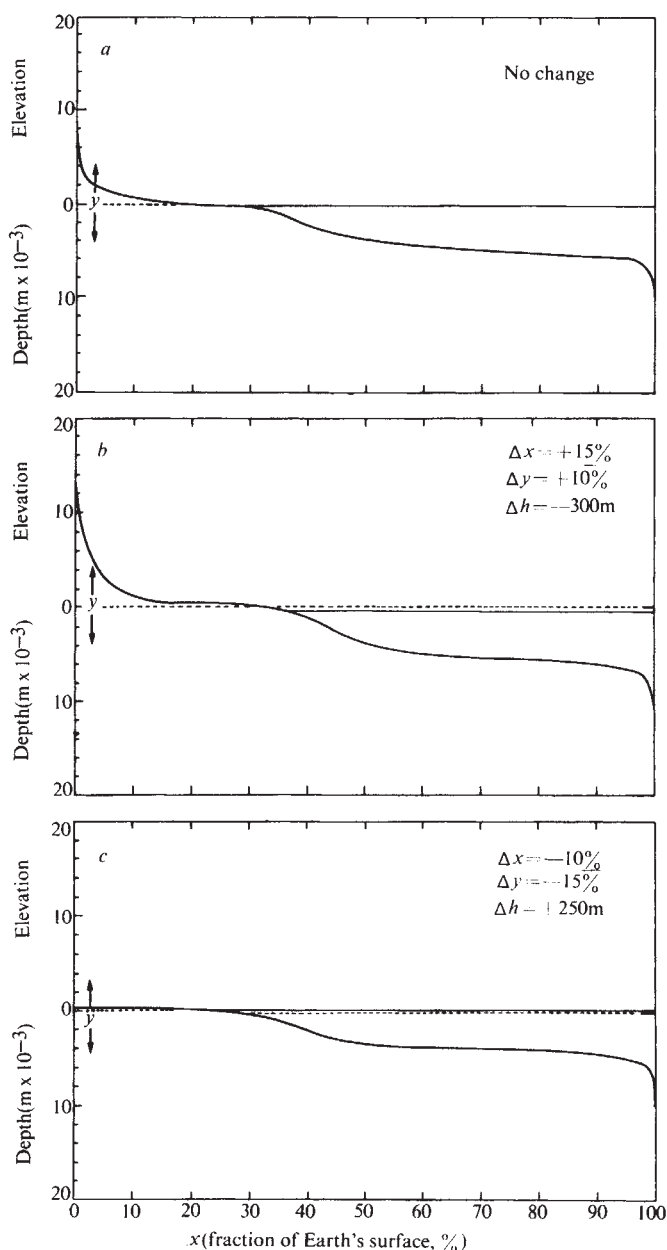


Fig. 1 a, The present hypsographic curve for the Earth^{16,17}. b, Hypothetical hypsographic curve after increasing continental area at present sea level by 15% and increasing ocean depth by 10%. Note increased height of continents, and decrease of sea level by 300 m. c, Hypsographic curve resulting from decreasing continental area at present sea level by 10% and decreasing ocean depth by 15%. Although continents are not reduced below present sea level, the eustatic rise in sea level results in total inundation.

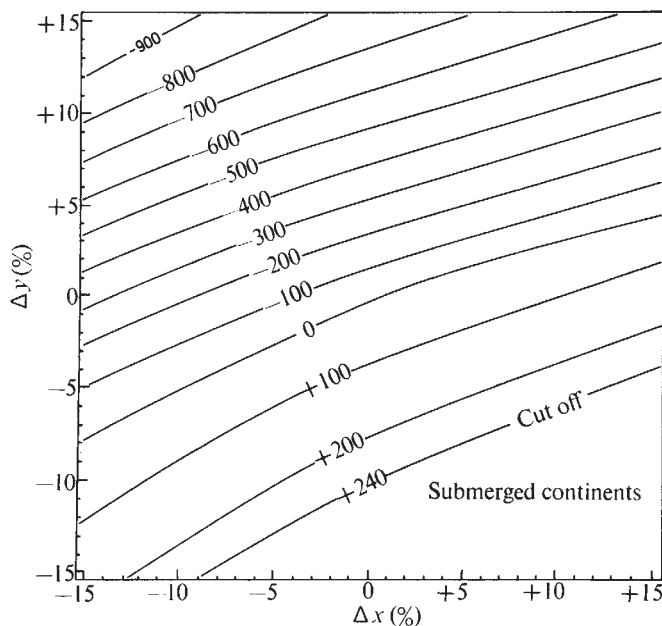


Fig. 2 Eustatic changes of sea level (m) relative to present sea level, contoured in terms of % change in continental area, x , and % change in ocean depth, y . For a rise of 240 m all continents are submerged.

such as continental margins, mid-ocean ridges and satisfying fault distributions observed by Van Andel and Heath¹⁸; horizontal dimensions were altered in the proportion new oceanic area/old oceanic area.

From comparison with a new hypsographic curve (Fig. 1) we estimated the change in solid volume of the Earth beneath the ocean. This change was added to or subtracted from the volume of the continents above present sea level, and the new profile of the continents calculated using rules of proportionality similar to those used for the oceanic profiles.

Two further boundary conditions are necessary and define the range of variability possible without causing total submergence or extreme altitude. Total submergence of the continents occurs if the postulated increase in Earth volume below the oceans is greater than the total volume of continents above present sea level. Large decreases in solid volume below the ocean, or large decreases in continental area, require excessive increases in continental altitude. Peak continental altitudes greater than twice the present value have been disregarded on isostatic grounds and because uplift is unlikely to exceed erosion by a sufficient amount.

Typical hypsographic curves for two extreme conditions are shown in Fig. 1. For each of the generated curves, the ocean area was drawn in with constant ocean volume and the sea level derived to an accuracy limited by scale to ± 50 m. The resulting changes in sea level varied from the maximum rise of 240 m to a maximum fall of 800 to 1,000 m. Errors of about $\pm 20\%$ were caused by difficulties in constructing the curves and measuring areas.

The model shows that small departures from the present proportion of continental area and ocean depth produce enormous changes in degree of flooding of continental margins (Fig. 2). For example, an extension of continental area results in a lowering of the mean height of continents, and an increase of land near present sea level with a necessary eustatic rise in sea level causing extensive transgression and shallow seas. An increase in continental area of 10% accompanied by a 6% decrease in mean ocean depth would submerge all continents.

Changes and Discontinuities

The oceanic part of a plate steadily subsides as it spreads away from the mid-ocean ridge axis^{3,19,20} and the aseismic

continental margin undergoes a similar thermal subsidence that declines exponentially with a time constant of about 50×10^6 yr (ref. 21). Transgressions and regressions on young margins may be masked by this effect and are therefore likely to be best observed on margins older than 50 m.y. We have therefore examined the transgressions and regressions shown on Sleep's generalized subsidence curve²¹ for the old, Triassic²² Atlantic margin of the United States for correlations with events in independently derived spreading histories^{23,24} for the North Atlantic Ocean (Fig. 3). Our comparison shows a good correlation between the Oligocene regression and deceleration in spreading at 45 m.y. and correlations between earlier eustatic changes and spreading discontinuities.

To check that this correlation does not reflect only the vertical movement of a particular plate, we have examined the Late Cretaceous and Cainozoic spreading histories of the major plates²⁵ for a global relationship. Global discontinuities in spreading have been postulated^{23,25-28}; our comparison (Fig. 4) shows a global deceleration in spreading at about 45 m.y., an acceleration at 10 m.y., and other discontinuities between 55 and 60 m.y. and about 70 m.y. Comparison of the ages of these discontinuities and eustatic changes documented on continental margins (Fig. 4 and Fig. 5) shows (within the limitations of the data) that the Oligocene-Miocene spreading deceleration is associated with a regression preceded by a minor transgression. The Late Miocene transgression just precedes the 10 m.y. acceleration and a transgressive-regressive phase may correlate with the 60 m.y. discontinuity. We therefore suggest that eustatic changes are connected with contemporaneous global spreading discontinuities and that older transgressions such as the Cainomanian will probably be associated with similar discontinuities.

A relationship between mid-ocean ridge elevation changes and eustatic changes has been suggested³⁻¹³ and the relationship between ridge elevation and spreading rate²⁹ has been supported by statistical evidence³⁰ showing that ridges spreading slower than 3 cm yr^{-1} tend to be higher than faster

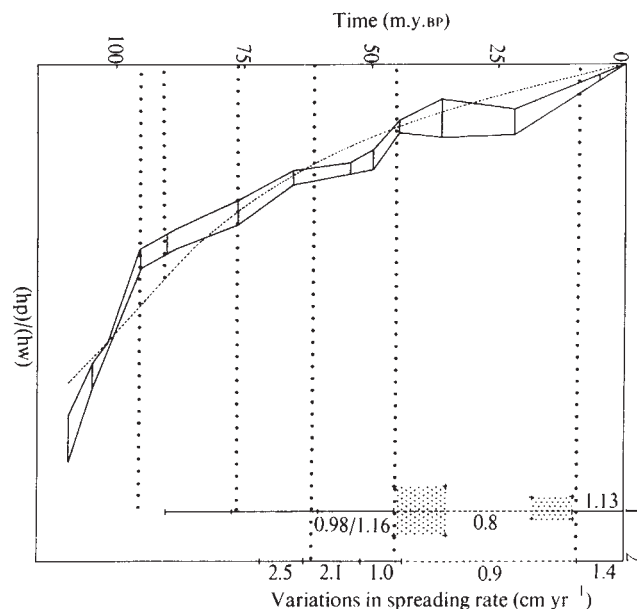


Fig. 3 Subsidence curve (---) for the Atlantic continental margin of the USA (from ref. 21). The depth normalized to the base of the Woodbine formation is plotted as a function of absolute age for wells on the East Coast. The error bars are 90% confidence limits. The subsidence curve is a 50 m.y. exponential constraint to fit data at the base of the Woodbine and, at the surface. Kinks in the data correspond to eustatic changes. Spreading history of the North Atlantic derived independently is shown in column 1 (ref. 23) and column 2 (ref. 24). . . ., possible correlations between eustatic change and spreading. Shaded area, transition from one spreading mode to another²³.

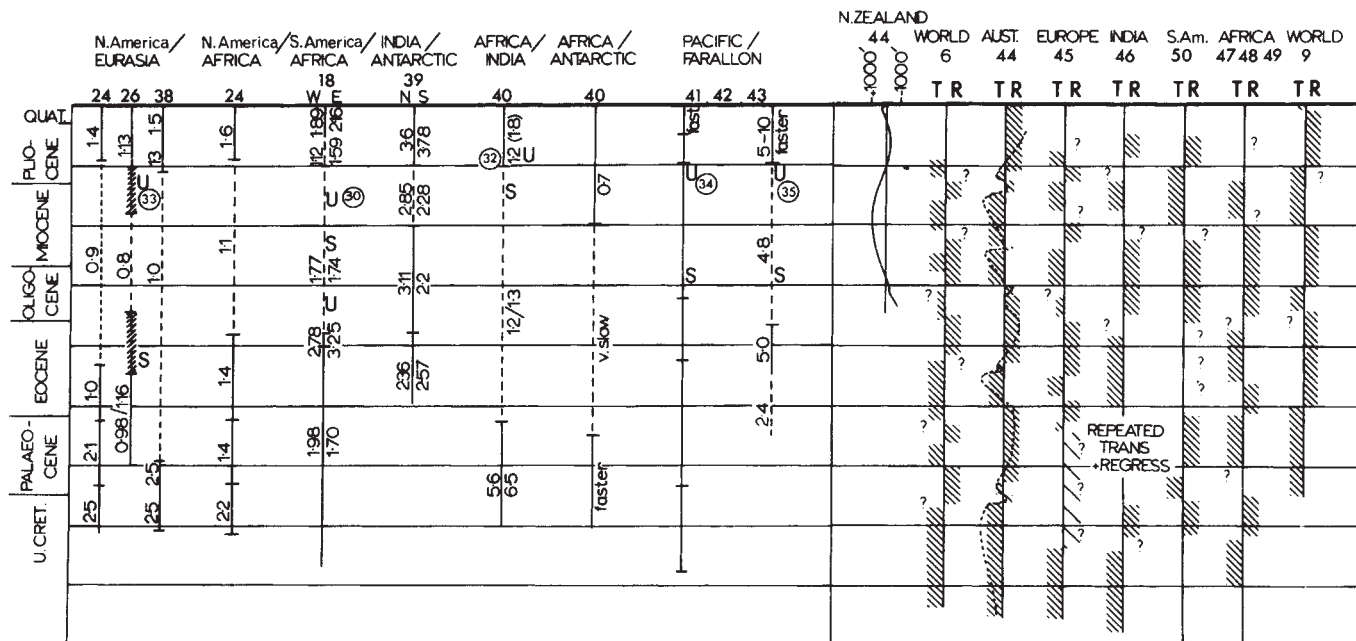


Fig. 4 Correlation between global discontinuities in spreading and global eustatic changes. ---, General reduction in spreading rate during the Oligocene and Miocene. Note correlation of uplift (U) and subsidence (S) with spreading rate changes and eustatic changes. Eustatic changes are shown as regressive (R) and transgressive phases (T) rather than curves showing interpreted amplitude of change. Circled numbers are references.

ones. Decelerations in spreading should therefore be accompanied by uplift and accelerations by subsidence. But deep-sea drilling data³⁰ and spreading rates¹⁸ for the southern Mid-Atlantic Ridge show the converse; the post 38 m.y. deceleration was followed by uplift in the Late Oligocene and subsidence in the Early Miocene; the ridge was uplifted to near its present elevation just before the 10 m.y. acceleration. Although these elevation changes correlate well with eustatic changes, the differences between the observed data and empirical model suggest that, at the slow (1.12 to 1.74 cm yr⁻¹) spreading rates on this ridge¹⁸, effects such as phase changes³¹ may also contribute to elevation changes. Other deep-sea drilling data³²⁻³⁵ can also be interpreted to support uplift and subsidence accompanying spreading rate changes. The transgressive-regressive sequence may be due to elevation changes during the adjustment period observed at some discontinuities²⁶.

General Implications

The paucity of deep sea drilling data does not permit us to estimate accurately eustatic changes caused by uplift and subsidence. But the southern Mid-Atlantic Ridge was uplifted³⁰ by between 500 and 1,000 m during the Late Miocene, a change of 25%. If we assume this uplift was not uniformly distributed over the South Atlantic¹⁸ and that uplift on other ridges was not perfectly synchronous, we can reasonably consider a reduction in ocean depth of about 10%. A depth decrease of this order would, provided the continental area remained constant, result in a eustatic rise of about 500 m (Fig. 2) because the change depends on the prior form of the hypsographic curve. If the continental area decreased during uplift, the eustatic change would be rather less but more if the area increased. Estimates of a Middle Miocene transgression of 350 m³⁶ suggest our calculations are of the right order.

The maximum eustatic rise of 300 m (including 60 m for deglaciation) implies uplift of all marine features now above 300 m. The maximal fall of 800 to 1,000 m indicates that sub-aerial processes may have influenced the morphology of the outer shelf, upper slope and canyons. Such a fall might have exposed sills in semi-enclosed seas resulting in circulation changes and possibly in complete evaporation; a lowering of this order could, for example, have contributed to the drying out of the Mediterranean in the Miocene³⁷ (see Fig. 2).

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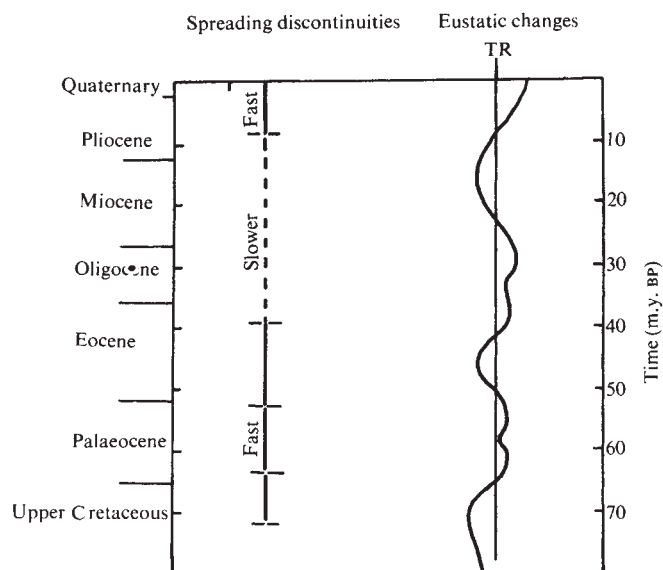


Fig. 5 Correlation between generalized global discontinuities in spreading and generalized global eustatic change (from Fig. 4). The eustatic change is necessarily approximate because of the limitations of the data.

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Resonance Raman Spectroscopy of the Photoreceptor-like Pigment of *Halobacterium halobium*

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Use of the resonance Raman technique has shown that the colour of the purple membrane pigment of *H. halobium* arises from an unprotonated Schiff base whose electron density is perturbed by further (electrostatic) interaction with a protein. The chromophore probably consists of a charge transfer complex between retinyllysine and an appropriate side chain of the protein.

RESONANCE Raman spectra have been reported for several molecules of biological interest, including haemoglobin^{1,2}, cytochrome C^{2,3}, rubredoxin⁴ and several carotenes⁵ and retinals^{6,7}. The resonant enhancement of certain Raman-active vibrations occurs when molecules are excited by light of a wavelength lying within an electronic transition⁸, and the effect provides a structural probe at unusually low solution concentration. Of particular importance to biological systems is the feasibility of *in situ* examination.

One potentially useful area of application for this technique is to photoreceptor pigments. The bathochromic shifts in visual pigments have not yet been satisfactorily explained. The absorption maxima of these pigments, in which there appears to be a Schiff base of 11-*cis* retinal with the ϵ -amino group of a lysine residue, vary from 430

to 562 nm (ref. 9). Solutions of the Schiff base itself, however, absorb near 360 or 440 nm, depending on whether the nitrogen atom is unprotonated or protonated, respectively. Thus another specific interaction occurs between protein and chromophore which perturbs the chromophore absorption band and gives rise to pigment colour¹⁰. Several theories proposed to explain visual pigment spectra have been reviewed by Abrahamson and Ostroff¹¹. The two most important ones are those of: (1) Morton *et al.*^{12,13}, which assumes that the primary bond is a protonated Schiff base in which the bathochromic shifts are provided by negatively charged groups appropriately positioned adjacent to the polyene chain; (2) the Dartnall formulation⁹, which assumes that the primary bond is an unprotonated Schiff base, with secondary shifts caused by an optimally placed pair of charges, producing massive dipoles in the polyene. Little experimental evidence is available with which to test either description.

Because of the anticipated extensive photochemical changes associated with the shining of intense laser radiation on a sample containing rhodopsin, I decided to examine initially the photoreceptor-like pigment of *Halobacterium halobium*. The "purple membrane" fragment containing the pigment offered several advantages for a laser-Raman spectroscopic study. (a) The primary structure of the chromophore is retinyllysine, the evidence being much the same as that for rhodopsin¹⁴. (b) Unlike rhodopsin, the pigment does not bleach or undergo photochemical changes on even prolonged exposure to intense (>200 mW) laser light, but simply undergoes a reversible spectral shift from $\lambda_{\max} = 558$ nm to $\lambda_{\max} = 570$ nm on exposure to red or blue light

respectively¹⁴. (c) The membrane is easily handled at room temperature in aqueous suspension.

The purple membrane used in the study described here was the gift of Drs D. Oesterhelt and A. E. Blaurock.

Cells of *Halobacterium halobium* were grown and the purple membrane fragments isolated as previously described¹⁴. Suspensions of membrane used for Raman spectroscopy had an optical density of 3.0 at $\lambda_{\max}$.

Raman spectra of purple membrane fragments excited with 4880 Å and 5145 Å radiation are shown in Fig. 1. The most intense vibration in each spectrum occurs at 1,531 cm^{-1} and about fifteen other peaks have also been distinguished. The only significant difference in the two spectra is the relative intensity of the 1,568 cm^{-1} vibration compared with that at 1,531 cm^{-1} . The former appears about four times more intense when excited by 4880 Å radiation than when excited by 5145 Å excitation. This observation is explained below.

Evidence for Resonance Enhancement

Several experiments showed that the observed spectra were resonance-enhanced. (a) The protein concentration as measured from the visible spectrum ($\epsilon_{\max}=54,000 \text{ l/mol cm}^{-1}$)¹⁴ was $5.5 \times 10^{-5} \text{ M}$ or two orders of magnitude less than that required to obtain ordinary protein Raman spectra¹⁵. (b) On bleaching the pigment with 0.1 M cetyltrimethylammonium bromide (pH 7.9), a procedure which leaves the Schiff base intact but destroys the purple colour¹⁴, no spectrum could be observed. (c) An excitation profile (variation of Raman intensity with excitation wavelength) experiment was carried out as follows. Samples of membrane were prepared containing 0.1 M Na_2SO_4 in which the non-resonant enhanced symmetric stretching vibration of the SO_4^{2-} ion at 983 cm^{-1} was used as an internal standard. The ratios of the intensities of the peaks at 1,531 and 1,568 cm^{-1} to the intensity of the standard were determined as a function of excitation wavelength

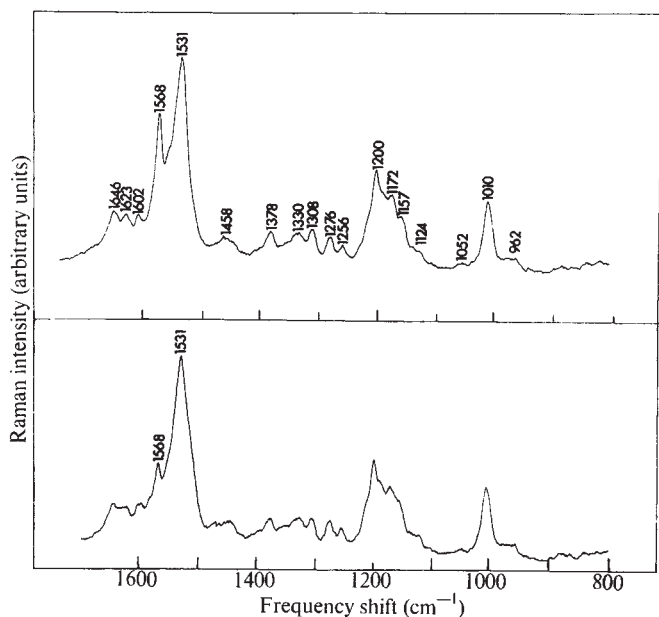


Fig. 1 Raman spectra of an aqueous suspension ($5.5 \times 10^{-5} \text{ M}$ in the purple protein, pH 7.0, unbuffered) of purple membrane fragments obtained with a 'Spex' 1401 Raman Spectrophotometer using: (Top) 100 mW of 4880 Å radiation from an Ar^+ laser. Resolution, 7 cm^{-1} ; time constant 2 s; scanning rate 25 $\text{cm}^{-1} \text{ min}^{-1}$; photon counting detection; 1 mm i.d. capillary cell, transverse excitation. (Bottom) 125 mW of 5145 Å radiation from an Ar^+ laser. Conditions as above. Polarization measurements indicated that all observed vibrations had depolarization ratios $0 \leq \rho \leq 0.3$. Spectra of membrane fragments suspended in 4 N NaCl solution are identical with those shown, as are spectra obtained in 0.05 M phosphate buffer (pH 7.0).

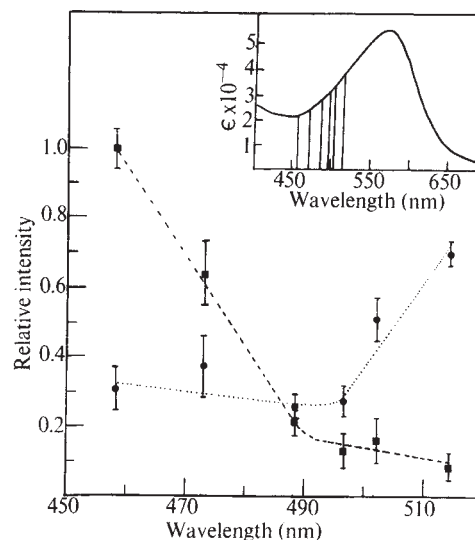


Fig. 2 Excitation profile results. ---, Ratio of the integrated intensities of 1,568 cm^{-1} vibration compared with 983 cm^{-1} vibration of SO_4^{2-} ion used as internal standard., Same ratio comparing the 1,531 cm^{-1} vibration with the standard. ■ and ●, Experimental points, observed at the indicated laser wavelength. Error bars represent standard deviations based on four measurements. Insert, pigment absorption band of purple membrane. Vertical lines indicate positions of laser lines located at 457.9, 472.7, 488.0, 496.5, 501.7 and 514.5 nm. The visible absorption spectrum was recorded on a 'Unicam' SP-800A spectrophotometer using cells of 2 mm path length.

using six lines available from the argon ion laser, all of which lie on the high frequency side of the pigment absorption band. The results are shown in Fig. 2. It is clear that there is substantial deviation from the $1/\lambda^4$ law for intensity of scattered light, as the observed ratios would not vary with wavelength if this relationship were obeyed. The two vibrations studied appear to be enhanced by (vibronic) coupling to different components of the membrane absorption spectrum. The peak at 1,531 cm^{-1} increases in relative intensity as the exciting line increases in wavelength toward the pigment $\lambda_{\max}$. It is therefore coupled to the main pigment absorption. The 1,568 cm^{-1} band, however, is increased in relative intensity as the wavelength is decreased, and it is therefore not coupled to the visible component of the membrane spectrum but to an absorption located at shorter wavelength than the main band.

All of the other vibrations (except that attributable to solvent O-H bending at 1,646 cm^{-1}) behave in a fashion similar to the 1,531 cm^{-1} vibration and hence arise from coupling to the 560 nm band of the pigment.

Analysis of Spectral Data

Spectral analysis is simplified by the fact that only chromophore vibrations are observed in the resonance Raman effect¹⁻³. Protein vibrations are of insufficient intensity to be seen and therefore do not complicate the spectra. In this work the region 1,500–1,700 cm^{-1} which contains C=C and C=N stretching vibrations will be considered. The region below 1,500 cm^{-1} , which contains C-C stretching and C-H bending modes, will be discussed in a future publication.

Rimai and coworkers have made a detailed Raman spectroscopic study of various retinals⁷ and their Schiff bases⁶. They found that those vibrational modes most strongly enhanced are contributed by C=C and C-C stretching in the conjugated chain, which occur near 1,570 and 1,200 cm^{-1} , respectively. In addition, they identified C=O and C=N stretching vibrations in the range 1,600–1,670 cm^{-1} .

In Fig. 3a and c, the 1,500–1,700 cm^{-1} region of the Raman spectra of unprotonated and protonated retinylhexylamine are shown. The C=N frequencies in this model Schiff base are expected to be similar to those of the pigment Schiff base

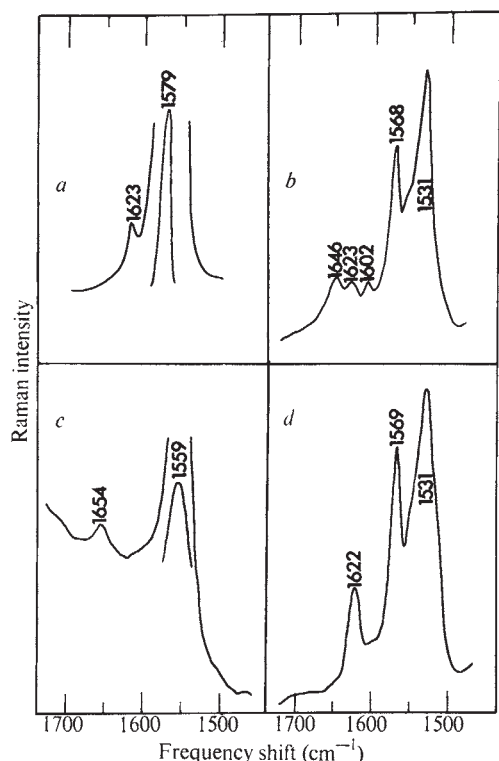


Fig. 3 1,500–1,700 cm^{-1} region of the Raman spectra of *a*, unprotonated retinylhexylamine (10^{-3} M in C_6H_{14} solution, $\lambda_{\text{max}} = 360$ nm); *b*, purple membrane fragments in H_2O suspension (see Fig. 1 (top), caption); *c*, protonated retinylhexylamine (10^{-4} M in acidified EtOH solution, $\lambda_{\text{max}} = 445$ nm); *d*, purple membrane fragments in D_2O suspension (conditions as in Fig. 1 (top) except for solvent). All spectra recorded using 4880 Å radiation. The difference in concentration in *a* and *c* reflects the increased resonant enhancement as the retinylhexylamine absorption band is shifted into the visible region. There is little or no change in the Raman frequencies of this Schiff base as a function of solvent. The best spectra of unprotonated and protonated retinylhexylamine were obtained in the solvent indicated.

and can be used to decide whether the nitrogen of the latter is protonated. In the unprotonated form (Fig. 3*a*), $\nu(\text{C}=\text{N})$ occurs as a weak band near $1,623\text{ cm}^{-1}$ while $\nu(\text{C}=\text{C})$ appears strongly at $1,579\text{ cm}^{-1}$. In protonated retinylhexylamine (Fig. 3*c*) the $\text{C}=\text{N}$ stretching frequency is broadened and shifted to $1,645\text{ cm}^{-1}$, while $\nu(\text{C}=\text{C})$ is shifted to $1,559\text{ cm}^{-1}$.

In Fig. 3*b*, the $1,500\text{--}1,700\text{ cm}^{-1}$ range of the purple membrane spectrum is shown. This region is complicated by the presence of a weak solvent OH bending vibration at $1,646\text{ cm}^{-1}$, although distinct peaks are seen at $1,531$, $1,568$, $1,602$, and $1,623\text{ cm}^{-1}$. The peak at $1,568\text{ cm}^{-1}$ is assigned to $\nu(\text{C}=\text{C})$ of the Schiff base. The excitation profile results described above indicate that this vibration arises from retinyllysine other than that in the pigment. Whether this free Schiff base is significant to the structure of the pigment is not clear. On suspension of the purple membrane in D_2O , the $1,646\text{ cm}^{-1}$ band disappears as expected, while the vibration near $1,623\text{ cm}^{-1}$ becomes more prominent (Fig. 3*d*) than in the H_2O preparation. This band, assigned to $\text{C}=\text{N}$ stretch of the Schiff base, appears at the same frequency as in unprotonated retinylhexylamine and strongly suggests that the Schiff base in the pigment itself is unprotonated.

The strongest peak in the purple membrane Raman spectrum at $1,531\text{ cm}^{-1}$ has no counterpart in the spectra of the model Schiff bases, all of which have intense $\text{C}=\text{C}$ stretching vibrations above $1,550\text{ cm}^{-1}$. The intensity of the $1,531\text{ cm}^{-1}$ band implies strong vibronic coupling to the pigment absorption and it is suggested that, by its magnitude and position, the vibration is still due to retinal $\text{C}=\text{C}$

stretch. The frequency decrease indicates that the π electron density of the conjugated system has been perturbed and electrons removed from the $\text{C}=\text{C}$ bonds. Such a process would reduce the $\text{C}=\text{C}$ stretching force constants and lower $\nu(\text{C}=\text{C})$.

The above observations indicate that pigment colour arises from an unprotonated Schiff base whose π electron density is perturbed by further (electrostatic) interaction with the protein. Additional evidence for this description was obtained when it was noticed that the purple membrane λ_{max} is highly solvent dependent (C. W. F. McClare, personal communication). Addition of a small amount of chloroform reversibly shifts λ_{max} of the pigment to 500 nm and the main Raman frequency to $1,520\text{ cm}^{-1}$. The chloroform seems to penetrate to the chromophore and thereby perturbs its electronic arrangement.

The exact geometry of the purple membrane pigment must await a three-dimensional structure determination; however, one model which this study points to is that of a charge transfer complex¹⁶ between retinyllysine and an appropriate side chain of the protein. Such interactions can account for the large shift in λ_{max} (from 360 nm to $\sim 560\text{ nm}$) of the chromophore as well as the perturbation of chromophore vibration frequencies upon complex formation. In addition λ_{max} in such complexes are quite solvent dependent¹⁶, as observed in the present case. That such a mechanism is feasible has been shown by Ishigami *et al.*¹⁷, who observed charge transfer between tryptophan and 9-*cis* retinal in acidified methanol solution (λ_{max} of complex = 520 nm). Furthermore, I have observed charge transfer between indole and *trans* retinal ($\lambda_{\text{max}} = 625\text{ nm}$) in the same solvent. It is therefore quite conceivable that an appropriately positioned tryptophan residue on the protein interacts with the π electrons of the Schiff base and produces the purple pigment colour.

A Raman spectroscopic study of rhodopsin and the intermediates present in its low temperature bleaching sequence would yield valuable information as to the nature of the Schiff base-protein interactions that determine pigment colour. The work described here illustrates the feasibility of such studies and the power of the resonance Raman technique in providing a structural probe for the photo-receptor pigments.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Search for Infrared Anomalies associated with Gravitational Events at the Galactic Centre

WEBER¹⁻⁴ has reported bursts of gravitational radiation apparently arriving from the direction of our galactic centre (or anti-centre). The resulting mass-loss implied by the estimated energy flux of nearly 100 W cm^{-2} at Earth has caused much theoretical discussion⁵⁻⁸ with regard to possible source models. This reported high gravitational flux at Earth suggests a search for accompanying electromagnetic bursts from the same direction. Such searches have already been made for microwaves^{9,10}, X-rays^{11,12} and neutrinos^{13,14}. All of these searches establish very small upper limits on the ratio of electromagnetic (or neutrino) to gravitational energy flux at Earth; however, radio pulses noncoincident with Weber's pulses have recently been reported¹⁵. Here we report the results of our search for bursts of infrared radiation from the galactic centre region.

Observations were made from the Cerro Tololo Inter-American Observatory, near La Serena, Chile. Clear nights provided continuous observation periods of up to 8 h. The infrared source coincident with the dynamical centre of the galaxy at RA 17 h 42–43 min and dec $-28^\circ 59'$ has recently been mapped at $2 \mu\text{m}$ and $10 \mu\text{m}$ (refs. 16–18). For observations of this source the 16-inch reflector was used to view a region one arc min in diameter at the centre of the infrared source. Observations covered a period from July 24 to August 8, 1972 (when weather permitted) and totalled 45 h of observing time. Observations were confined to those times when the galactic centre was near the zenith. These times also correspond theoretically to a maximum in the response of an east-west oriented gravitational antenna.

We used a liquid nitrogen cooled PbS detector to view the $2 \mu\text{m}$ and $4 \mu\text{m}$ atmospheric windows. Before each observing period, a scan of $\alpha \text{ Sco}$ was made as a calibration of detector noise and sensitivity. The galactic centre infrared source is first magnitude^{16,17,18} in the L band and could be seen with a 2:1 signal-to-noise ratio using a 100 s time-constant. The chart recorder monitor using a 0.1 s time-constant could easily have detected an increase of two orders of magnitude in infrared flux from the galactic centre.

Weber has supplied us with a list of 74 gravitational events covering the period July 23 to August 8, 1972. During our observations, Weber recorded five coincidences between gravitational antennas at the Maryland and Argonne Laboratories. No significant increases in infrared flux above a level of $10^{-14} \text{ W cm}^{-2}$ were found within $\pm 1 \text{ min}$ of these events. This upper limit on the infrared flux that might be associated with a gravitational pulse is a factor of 10^{16} less than the gravitational flux necessary to excite the coincidences in the gravitational antennas. Since the bandwidth of the $2 \mu\text{m}$ and $4 \mu\text{m}$ windows is $2 \times 10^{13} \text{ Hz}$, the energy flux per unit bandwidth in the infrared fluctuated less than $5 \times 10^{-28} \text{ W cm}^{-2} \text{ Hz}^{-1}$ during a gravitational event. This limit is of the order of 10^{28} less than the required gravitational flux per unit bandwidth and is

nearly the same as the limit set in the microwave regions^{9,10,15}.

If the reported high flux gravitational events are confirmed, there are several possible explanations for this absence of observed coincident fluctuations in infrared levels. First, if there is a net polarization of the gravitational radiation, it has been shown that the location of the source of gravitational radiation is not near the galactic centre region^{6,7}. Second, dust in the dense region within 1 pc of the galactic centre may absorb most electromagnetic radiation, re-emitting it over comparatively long time periods. Third, there may simply be very little electromagnetic radiation accompanying such gravitational events. Finally, if one assumes no net polarization of the gravitational waves, Weber's data only limit the position of the source to within 4 arc deg of the galactic centre. We have narrowed this search to the small region near the galactic nucleus because the high mass density there makes gravitational radiation more plausible.

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Position of OH471

Carswell and Strittmatter, reporting the high redshift¹ they measured for OH471, do not quote the accurate radio and optical coordinates which were the basis of its identification. These coordinates are:

Radio $\alpha(1950) = 06 \text{ h } 42 \text{ min } 53.3 \text{ s} \pm 0.2 \text{ s}$ $\delta(1950) = 44^\circ 54' 33'' \pm 1''$
Optical $\alpha(1950) = 06 \text{ h } 42 \text{ min } 53.1 \text{ s} \pm 0.1 \text{ s}$ $\delta(1950) = 44^\circ 54' 31'' \pm 1''$

The radio position was measured by the method described by Adgie *et al.*² except that an interferometer spacing of only 1,300 wavelengths was used. The optical position was meas-

ured on the prints of the Palomar Sky Survey relative to AGK2 stars: the accuracy achieved was estimated from the distribution of radio-optical position differences obtained in a large number of similar measurements.

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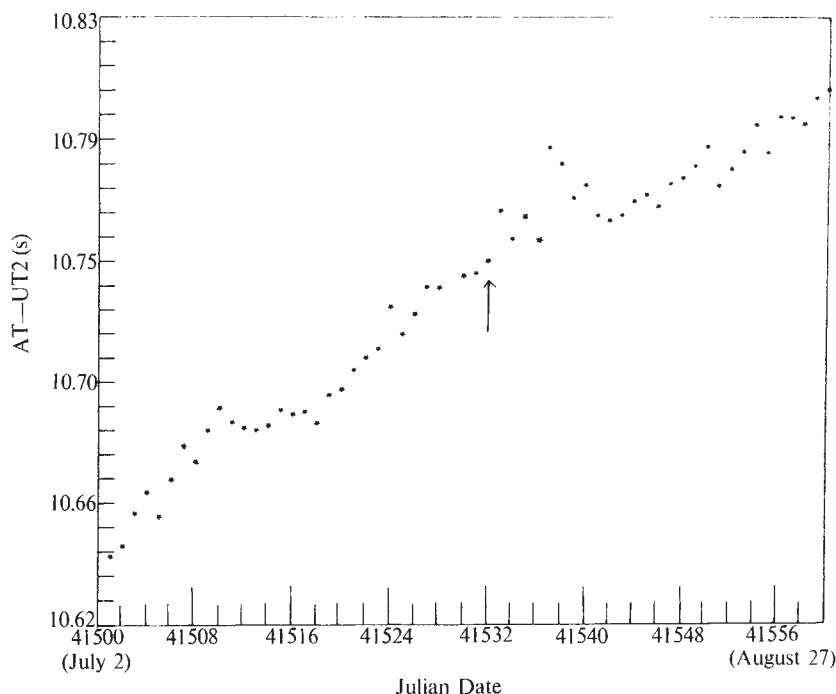
Discontinuous Change in Earth's Spin Rate following Great Solar Storm of August 1972

THE question of a link between changes in the Earth's spin rate and the activity of the Sun is of topical interest, and there is good evidence that the changing length of day is influenced by the mean level of solar activity^{1,2}. The possibility of a one-to-one correlation between specific events on the Sun

length of day, and thus in the spin rate of the Earth, are revealed by regular measurements of Universal Time (UT) carried out at many observatories around the world. For our purpose, we are interested in UT2, the version of Universal Time with the effects of the Chandler Wobble and seasonal variations removed. The difference between Atomic Time (AT) and UT2 shows, on average, a monotonic increase as the Earth's spin slows down and the length of day increases.

In Fig. 1 we plot AT-UT2 for a period of one month on either side of the great solar storm of August 1972. The spot group associated with the flare activity built up from July 29, reaching a maximum size (covering 17° in longitude) on August 4. In Fig. 1, we have marked August 3 (JD 41532), the last day before the series of Forbush decreases which marked the solar activity⁷, by an arrow. The expected change in spin rate and length of day is clearly visible; a similar plot using data for the six month period May to October 1972 shows (Fig. 2) that the jump is the largest such event recorded over that period. The change in slope indicated by the data of Fig. 2 is equally significant; this is qualitatively similar to the changes seen in pulsar spin rates during glitches, and we therefore borrow the term from the pulsar literature to describe this terrestrial process. At the time of the flare and sunspot activity, the slope flattens

Fig. 1 Change in length of day for a period of one month on either side of date of great solar flare activity of August 1972. Arrow marks August 3, the last day before the flare activity. Large discontinuous change in AT-UT2 occurs on August 8.



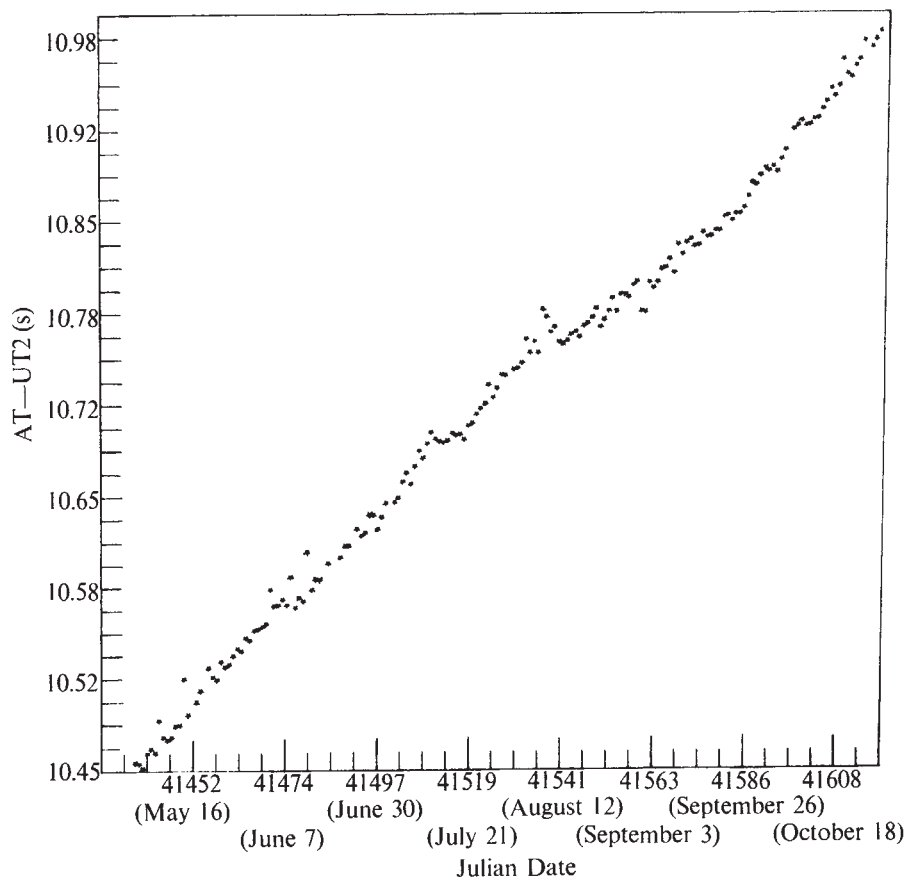
and specific changes in the length of day has remained more controversial, however, although there was a suggestion of such an effect associated with the great solar storm of 1959 (refs. 3-5). Specifically, Danjon suggested³⁻⁵ that there was an increase in the length of day when the nucleonic component of solar cosmic rays increased; this was in addition to the usual steady increase in the length of day. Other observers questioned the reality of this effect (for a discussion of the controversy see ref. 6), and because the 1959 solar storm was the greatest recorded since the time of Galileo, there was no immediate hope of an independent test of Danjon's claim. In August 1972, however, an even greater disturbance occurred on the Sun⁷⁻⁹. It seemed to us that this might provide the ideal opportunity to resolve the controversy, and we have indeed found a discontinuous change in the length of day, and a change in the rate of change of the length of day (a glitch) immediately after that event. Changes in the

slightly, subsequently trending back towards a slope more typical of the data of the preceding three months over a period of several weeks.

These effects are not so dramatic that one would necessarily attribute them to an outside cause on the basis of these data alone, but they take on a greater significance in the light of our prediction, following Danjon, that just such a change should occur soon after a great solar flare. We are confident that the effect is real, and that the glitch was indeed caused by events associated with the solar activity of early August 1972.

It is not difficult to envisage models which explain the delay of 5 days between commencement of the flare activity and the glitch. We will not discuss detailed mechanisms here, except to point out that solar phenomena are known to influence the large scale circulation of the Earth's atmosphere. For example, troughs in the circulation pattern at high latitudes are amplified when the level of solar cosmic rays reaching

Fig. 2 As Fig. 1, for May to October 1972. The change in slope after the time of the great solar activity, and subsequent return towards the pre-flare slope, emphasize the importance of the event.



the Earth is high^{10,11}. Like Schatzman⁶, we believe that sudden variations in the length of day may be produced by meteorological phenomena induced by solar activity; in that case, it would be most unreasonable if it did not take a few days for these effects to show themselves in the AT-UT2 measurements.

We will discuss details of such a mechanism and further consequences of this discovery elsewhere. We thank the US Naval Observatory, Washington, for supplying the raw data used in the preparation of Figs. 1 and 2.

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Viscous Remanent Magnetization in Oceanic Basalts

THE magnetic properties of the basalts which form layer 2 of the oceanic lithosphere are important because of their relevance to the hypothesis¹ of seafloor spreading. Most studies of these magnetic properties have been carried out on basalts obtained from dredge hauls taken predominantly from ocean ridge systems and fracture zones. These constitute special areas of the oceanic crust where the sediment cover is negligible. It is of interest to compare the magnetic properties of the dredged basalts with samples recovered from holes drilled through the overlying sediments into the basaltic layer at places distant from ridge axes. Samples obtained from the abandoned Mohole project and, more recently, from the Deep Sea Drilling Project (DSDP) possessed magnetic properties similar to those of dredged basalts^{2,3}. Here I describe highly unstable magnetic characteristics found in basalts from DSDP hole 57.

In a study of basalts from DSDP holes in the Atlantic Ocean³ the natural remanent magnetizations (NRM) were found to be very stable, requiring alternating fields of 200–400 oersted to destroy 50% of the original remanence. The NRM directions and intensities were invariant in storage tests in the Earth's magnetic field lasting several months. High Königsberger ratios, averaging about 11, imply that the remanent magnetizations were much more important than magnetizations isothermally induced by the ambient geomagnetic field. The remanent intensities were considerably lower than those found in dredged basalts (1×10^{-3} gauss compared to about 5×10^{-3} gauss), but were strong enough to account for the observed magnetic anomalies if a thickness of 2.5 km is assumed for a uniformly magnetized layer 2. Palaeolatitudes deduced from the remanent inclinations of extrusive basalts agreed with palaeolatitudes derived from a model reconstruction of the opening of the Atlantic Ocean⁴. The basalt magnetic pro-

perties were therefore compatible with the Vine and Matthews hypothesis¹.

Present investigations of DSDP basalts from several holes in the Pacific Ocean have shown that their magnetic properties are similar to those described above and to those of dredge haul samples (W. L., R. Løvlie and N. D. Opdyke, unpublished). But the remanent inclinations were different from those expected at the sites. The inclinations of partially oriented oceanic basalts have been found shallower than anticipated in other studies^{2,5-7}. Otherwise the magnetic character of tholeiitic basalts seems to satisfy the demands imposed by interpretations of marine magnetic anomalies in terms of seafloor spreading. But very unstable magnetic behaviour found in hole 57 basalts contrasts markedly with the usual high stability.

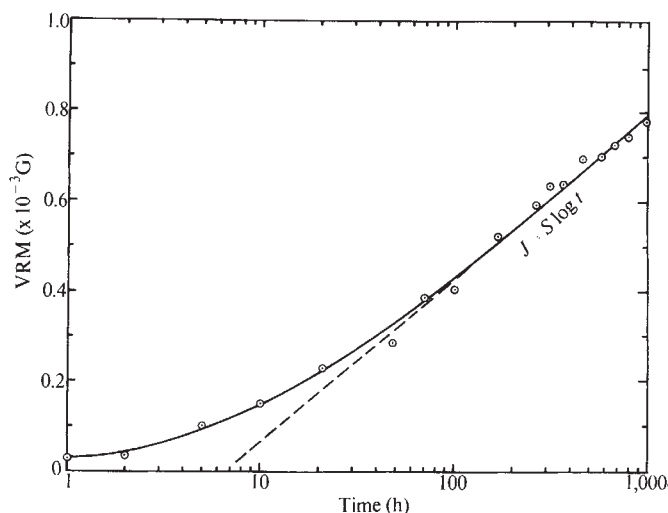


Fig. 1 Acquisition of VRM in a specimen from DSDP hole 57. Core 2-2, depth 60 cm, $S = 0.34 \times 10^{-3}$ gauss.

Hole 57 was drilled at $8^{\circ}41' \text{ N}$, $142^{\circ}32' \text{ E}$, on the north flank of the Caroline ridge and to the south-east of the Marianas trench. The overlying Late Oligocene sediments were unaltered and the basalt was initially identified as a thick flow (or "lava lake") rather than a sill⁸. Subsequent analysis has given a K/Ar age of 23.5 m.y. for the basalt (I. Ridley *et al.*, unpublished). The basalt is more alkaline than the usual ocean ridge tholeiitic basalt, and is of a type associated with oceanic islands and island chains⁶.

The basalt texture was doleritic, by contrast with the fine-grained textures usually found in chilled oceanic basalts. About 20% consisted of clots of large euhedral plagioclase crystals, with a matrix consisting of "ophitic areas con-

taining olivine granules, and patches of quench material rich in oxide skeletal crystals"⁹. From an examination of polished sections with a reflected light microscope, R. Løvlie categorizes the deuteric oxidation state of the basalt as class I, according to the classification scheme of Wilson and Watkins¹⁰. About half of the skeletal, homogeneous titanomagnetite grains were finer than $10 \mu\text{m}$ to $200 \mu\text{m}$. More usually, titanomagnetite grains in oceanic basalts are less than $5 \mu\text{m}$ in diameter, and often are too fine for internal features to be resolved by conventional optical microscopy. The Curie temperatures of the specimens averaged 150° C , some 100° C lower than the values found in other DSDP basalts from the Atlantic and Pacific oceans³. This indicates that the titanium content of the titanomagnetites was probably higher in the basalts from hole 57, in agreement with observations based on other petrologic evidence.

The remanent magnetizations were measured with a spinner magnetometer. Alternating fields averaging only 40 oersted were capable of destroying 50% of the NRM of each specimen. The magnetic coercivities were so low that adequate demagnetization was impossible. The minimum dispersion of the remanent inclinations occurred after demagnetization in a peak field of 150 oersted. The mean inclination was then $16^{\circ} \pm 4^{\circ}$ (standard error), which was not significantly different from the axial dipole field inclination (17°) at the latitude of the site.

After demagnetization in fields of only 150 to 200 oersted the magnetization was observed to change rapidly while it was being measured, apparently because of the decay of very soft viscous remanent magnetization (VRM) components. Similar viscous magnetic behaviour has been reported in DSDP basalts from the southern Caribbean⁶.

Eight of the unstable specimens were demagnetized in peak alternating fields of 300 oersted, and placed in a field-free space for 2 days. A field of 0.53 oersted was then applied in a known direction relative to each specimen. The remanences were measured at intervals, spaced approximately logarithmically, for 1,000 h (6 weeks). In each specimen the remanent magnetization slowly increased in intensity and its direction approached that of the applied field. The acquisition of this VRM followed the same pattern in each case (Fig. 1). Initially the acquired VRM interacted with the remaining part of the NRM, but after 50 to 100 h the growing VRM dominated the process and the magnetization (J) increased logarithmically with time (t) as $J = S \log t$, which is characteristic of VRM¹¹⁻¹⁴.

The magnetic viscosity coefficient, S , was measured for each specimen from the slope of the linear portion of each semilogarithmic graph. These values for S gave the intensities for the VRM that could have been acquired since the time of the last well-established reversal of the geomagnetic field, 0.69 m.y. ago (Table 1).

The VRMs acquired in 1,000 h were next demagnetized

Table 1 Measured Values of the Magnetic Viscosity Coefficient (S), the VRM Acquired in 1,000 h in a 0.53 Oersted Field ($\text{VRM}_{1,000}$), and the Alternating Field Required to Erase 90% of that VRM ($H_{1,000}$)

Specimen No.	S	Magnetization ($\times 10^{-4}$ gauss)		NRM	Demagnetizing field (oersted)		
		$\text{VRM}_{1,000}$	$\text{VRM}_{0.69\text{M}}$		$H_{1,000}$	$H_{0.69\text{M}}$	H_{NRM}
57-2-1-83A	3.2	8.3	30	42	59	190	107
57-2-1-113A	3.9	9.2	36	24	32	105	125
57-2-1-148A	3.2	7.9	30	69	40	130	95
57-2-1-148C	2.8	7.5	26	32	38	125	150
57-2-2-48A	3.0	7.3	28	69	109	355	100
57-2-2-60A	3.5	7.9	32	33	137	450	130
57-2-2-149A	3.9	9.3	36	28	42	135	130
57-3-1-12A	3.5	8.7	32	26	53	170	120

Computed values of the VRM that could be acquired in 0.69 m.y. ($\text{VRM}_{0.69\text{M}}$) and the field to erase that remanence ($H_{0.69\text{M}}$) are shown in comparison to the NRM intensity and its 90% erasure field (H_{NRM}).

progressively in alternating magnetic fields. From the demagnetization curves the field ($H_{1,000}$) required to destroy 90% of the VRM was determined. The field needed to destroy a VRM is proportional to the logarithm of the acquisition time^{15,16}. Therefore, it is possible to deduce the field ($H_{0.69M}$) that would be required to destroy a VRM acquired since the beginning of the Brunhes normal polarity epoch from $H_{0.69M} = H_{1,000} \times \log(0.69 \text{ m.y.}) / \log(1,000 \text{ h}) = H_{1,000} \times 3.26$ (see Table 1).

The data indicate that both the intensity and the stability of the natural remanent magnetization of the hole 57 basalt can be accounted for by a magnetization which is almost entirely viscous, acquired since the beginning of the Brunhes epoch. Viscous remanences such as these, requiring alternating fields of one or two hundred oersted for erasure, have previously been reported in other titanomagnetite-bearing igneous rocks¹⁶. It is interesting that in spite of the viscous nature of their remanences the samples had Königsberger ratios ranging from 2 to 7, contradicting the suggestion that ratios greater than 1 in igneous rocks imply single domain thermoremanent magnetization¹⁷. The presence of an appreciable coarse-grained titanomagnetite fraction suggests that the remanence is a multidomain type VRM.

In the Brunhes epoch there are several postulated short reversals, some of which may only represent excursions of the geomagnetic field rather than true reversals¹⁸⁻²². Some of these short events may be different recordings of the same event. Their durations are thought to be only 2,000-6,000 yr. The effect on the intensity of VRM that may have been acquired during the predominantly normal epoch would be to reduce it by only a few per cent, and their possible existence does not alter drastically the computed VRM for that period of time.

Available information for oceanic ridge systems that have associated linear magnetic anomaly patterns indicates that the underlying basalts possess stable magnetic properties that account for these anomalies. Most submarine basalt magnetic studies have been carried out on fine-grained ocean ridge tholeiites obtained from dredge hauls or deep sea drilling. The unstable character of the basalt from hole 57 is quite different from that found in ocean ridge basalts. The basalt is also different in that it is probably an oceanic island type (I. Ridley *et al.*, unpublished), more differentiated than ocean ridge tholeiites. The iron oxides have a slightly higher titanium content than usual and are coarser in grain size.

The basalts that form oceanic islands, island chains, seamounts and ocean bottom lava flows arising from volcanic activity distant from ridge systems are genetically different from oceanic ridge tholeiites. If the hole 57 basalt is typical of this group then unstable magnetic behaviour may be more common in oceanic basalts than previously supposed. Unstable and very low coercivity NRM has been observed in other DSDP basalts from the Eastern Atlantic⁵ and VRM has been detected in DSDP basalts from the Caribbean Sea⁶. Where such basalts form the oceanic basement magnetic anomalies would only reflect topographic influence and local variations in magnetization contrast. A correlatable linear magnetic anomaly pattern would be absent.

Several explanations have been offered for the existence of regions of uncorrelatable magnetic anomalies, or quiet zones²³, and it may be that no unique explanation will serve for all these areas. None of the explanations is based upon measured rock magnetic properties. The results of this study of the magnetic instability in hole 57 basalts suggest another possible explanation which may be applicable to some quiet zones. The lack of significant anomalies in these quiet zones may be due to viscous remanent magnetization of the underlying crust.

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Comments on "Is the African Plate Stationary?"

Burke and Wilson¹ suggested that the African plate became stationary some 25 m.y. ago after having moved northeast from the Mid-Atlantic ridge since the opening of the South Atlantic. Like Rhodes² they believe that the continent drifted over hot spots or convective plumes in the mantle and that Walvis Ridge and the Discovery and Meteor seamount chains represent trails of hot spots marking the relative motion of Africa.

The west coast of Southern Africa is characterized by linear belts of plutonic and volcanic complexes; if these rocks were generated over plumes and the African plate has moved laterally over them they should appear progressively younger in the same direction, from east to west.

The hypothesis may therefore be tested by examining the isotopic age data for the lineaments. This has been done by Marsh³ for the Upper Mesozoic alkaline igneous complexes of Angola and South West Africa (Fig. 1), demonstrating that no sensible pattern in the variation of ages exists. The available data range from 123 m.y. to 164 m.y. (refs. 4, 5) and do not support the mantle plume theory. Similar results were obtained for two E-W lines of alkaline complexes in Brazil³ where the ages range from 132-80 m.y. and 80-51 m.y. respectively⁶, showing no directional pattern where, according to the

hot spot hypothesis, younger ages should be expected from W to E.

Instead, Marsh³ correlated the lineaments of alkaline igneous activity with transform faults now active, offsetting the Mid-Atlantic ridge, and concluded that the Upper Mesozoic complexes reflect the initial rifting of the Africa-South America plate some 130 m.y. ago.

The oldest lineament of igneous activity along the west coast of southern Africa consists of the Kuboos-Tatasberg-Bremen-Garub line (Fig. 1) and is lower Palaeozoic. The Kuboos Granite was dated at 550 ± 20 m.y. (ref. 7) and provisional data indicate an age of 560 m.y. for the Bremen complex further northeast. A pyroclastic sill associated with the carbonate-bearing diatremes at Garub yielded 500 ± 5 m.y., but it is uncertain whether this age represents the time of extrusion or a later regional thermal event (A. K., H. Allsop and H. Welke, unpublished). Nevertheless, no systematic change in age following the 250 km long lineament is apparent.

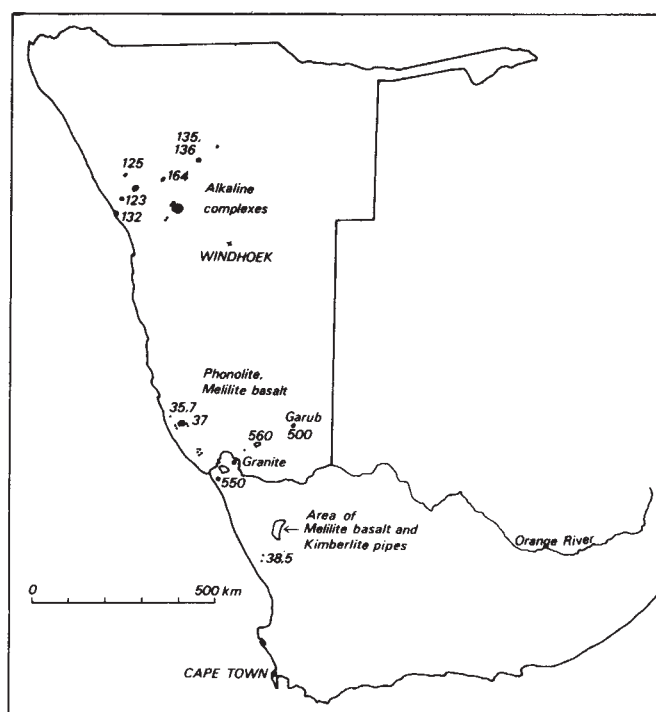


Fig. 1 The early Palaeozoic, Upper Mesozoic and Tertiary lines of volcanic complexes along the west coast of southern Africa. Numbers are radiometric ages in m.y.

Burke and Wilson¹ maintain that such surface structures can only be related to those of the underlying asthenosphere if a plate is temporarily at rest. The alkaline complexes of South West Africa clearly follow a NE trend which reflects the regional structures of the underlying Palaeozoic Damara Orogen, yet the African plate had already begun to drift at the time of alkaline igneous activity^{8,9}.

During the Oligocene the coastal areas of southern South West Africa and Namaqualand were affected by volcanic activity over a length of some 450 km (Fig. 1). The lines of phonolite and melilit basalt plugs are clearly oriented in a NNW direction and follow structural trends of Late Precambrian origin. Their ages range between 35.7 and 38.5 m.y. (J. A. S. Adams, personal communication) when, according to Burke and Wilson, the African plate was still in motion.

Clearly, lines of volcanic activity in southern Africa are related to older structures in the crust without the African plate necessarily being at rest. I believe that none of the lines mentioned here represents trails of hot spots and I am not

aware of any line of volcanoes in Africa that shows a consistent age pattern in any direction.

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Removal of Xenon and Radon from Contaminated Atmospheres with Dioxygenyl Hexafluoroantimonate, O_2SbF_6

RADIOACTIVE noble gases, such as ^{133}Xe , ^{135}Xe , ^{85}Kr , and ^{88}Kr , are formed in uranium fission and are released to the atmosphere by nuclear power plants and fuel reprocessing plants. Studies of the US Public Health Service have shown that a modern boiling-water reactor, for example, releases about 20 Ci of ^{85}Kr and 13,000 Ci of ^{133}Xe per year under normal operating conditions^{1,2}. Under adverse operating conditions, the release rates may be much higher¹. Of the physical methods for reducing these emissions³⁻⁷, cryogenic distillation and charcoal adsorption are being developed for large-scale use.

Soon after the first noble gas compounds had been discovered⁸⁻¹², Pomeroy¹³ suggested that chemical methods might be useful for trapping krypton and xenon isotopes. Slivnik¹⁴ has studied reactions of krypton-xenon mixtures with fluorine for waste-gas treatment and has demonstrated that xenon can be separated from krypton by such reactions. Fluorine is not, however, a convenient reagent for this purpose, as it must be heated with the process gas, and the excess fluorine must be removed afterwards. I have shown that radon, the heaviest noble gas, can be collected by oxidation with liquid bromine trifluoride and solid halogen fluoride-metal fluoride complexes, such as ClF_2SbF_6 , BrF_2SbF_6 , BrF_2BiF_6 , and $IF_4(SbF_6)_3$ (refs. 15 and 16). I report here further experiments with a dioxygenyl salt, O_2SbF_6 , which reacts with radon and xenon at 25° C and which appears very promising as a reagent for removing both of these gases from contaminated atmospheres.

Dioxygenyl hexafluoroantimonate was prepared in 0.5–10 g amounts by photochemical reaction of oxygen, fluorine, and antimony pentafluoride¹⁷. Samples (0.5 g) of the compound, a white, crystalline powder, were exposed to samples of radon, xenon and krypton in 300 ml. 'Pyrex' bulbs on a metal vacuum line. Tracer ^{222}Rn (8.1 mCi) reacted with the powder immediately at 23–25° C, forming a nonvolatile radon compound. Xenon at 10–200 mm pressure also reacted immediately, forming a pale-yellow, translucent xenon compound. Oxygen was shown to be liberated in the xenon reaction by the fact that the residual gas was not completely condensable at –195° C. No reaction of krypton at 485–700 mm pressure was observed from 23° to 150° C.

Flow experiments were carried out with O_2SbF_6 powder and samples of air (0.33–0.76 l. at STP) which had been artificially contaminated with ^{222}Rn and ^{133}Xe . In each experiment, a rare gas/air mixture was passed through a glass U-tube packed with the powder, then through a trap cooled with liquid

nitrogen to condense any unreacted radioisotope. The distribution of the radioisotope was afterwards determined by measuring the γ -emission of the U-tube and the cold trap. (The distribution of ^{135}Xe was determined immediately; the distribution of ^{222}Rn was determined after 3 hours, when ^{222}Rn and its γ -emitting daughters ^{214}Pb and ^{214}Bi were known to be in radioactive equilibrium.) In three experiments with the radon isotope and with a bed of powder 5.0 cm long and 6.3 mm in diameter, all of the radon was absorbed. In 5 experiments with the xenon isotope and with a bed of powder 6.5 cm long and 5.5 mm in diameter, 67–100% of the xenon was absorbed (Table 1).

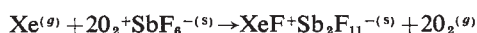
Table 1 Removal of Radon and Xenon from Air with O_2SbF_6 at 23–25°C

Radioisotope	Conc. in air (mCi l. ⁻¹)	Average flow rate (ml. min ⁻¹)	Amount of radioisotope removed (%)
^{222}Rn	13	12	100
"	15	15	100
"	24	12	100
^{135}Xe	9.8	15	67*
"	7.9	13	100
"	2.0	14	100
"	3.2	13	98
"	4.3	14	100

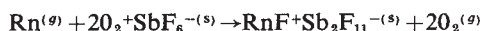
* Flow rate poorly controlled at start of experiment.

Raman spectral studies have shown that the xenon product is $\text{XeF}^+\text{Sb}_2\text{F}_{11}^-$, a 1:2 xenon difluoride-antimony pentafluoride complex^{18–20}. When xenon is added gradually to O_2SbF_6 , a new band (characteristic of the xenon-fluorine stretching vibration of XeF^+ cation¹⁵) appears in the spectrum at 618 cm⁻¹. Shifts in the SbF_6^- vibration frequencies also occur. The final spectrum contains prominent bands at 450, 618, 655, and 686 cm⁻¹.

Mass spectrometric analyses of residual gases in experiments with krypton-xenon mixtures have shown that two molecules of oxygen are released for each atom of xenon absorbed as follows



No spectral or analytical data have been obtained for the trace amounts of radon product (no stable isotopes of radon are known, and the ^{222}Rn product is intensely radioactive), but radon probably forms an analogous 1:2 radon difluoride-antimony pentafluoride complex as follows



Mixtures of approximately three parts O_2SbF_6 and one part antimony pentafluoride by volume (grey powders, prepared in a dry box) react with radon but not with xenon. Dilution with antimony pentafluoride therefore lowers the free energy of O_2SbF_6 below the threshold for xenon oxidation but not below that for radon oxidation.

Equimolar mixtures of krypton and xenon were partly separated on passage through U-tubes packed with O_2SbF_6 . The mixtures were passed through beds of the compound 5.8–6.0 cm long and 5.5 mm in diameter at 23–25°C and at total pressures of 20–160 mm. Gases emerging from the beds were collected and analysed by mass spectrometry. The first samples had the following range of composition: 45.5–49.6% O_2 , 12.7–16.2% Xe, and 37.7–38.3% Kr. The percentage of oxygen fell and that of krypton and xenon rose as the O_2SbF_6 was depleted. The colour change of the powder, from white to yellow, could be used to indicate the amount depleted, as there was a sharp interface between coloured zones. Each time that gas was admitted, a blue-green fluorescence was noted in the O_2SbF_6 . The source of this emission is not known at present, but it is probable that an excited xenon, oxygen, or ozone species is formed during the reaction.

More complete separation of krypton and xenon was achieved by shaking equimolar mixtures of the gases with O_2SbF_6 powder in 'Pyrex' bulbs containing stirring vanes. The mixtures were shaken intermittently, by hand, at room temperature with excess amounts of the powder over periods of 3 to 24 h. Residual gases were then analysed by mass spectrometry, and the final gas mixtures were found to contain less than 2% xenon.

Because it has negligible vapour pressure at 25°C, and because oxygen is the gaseous product, the dioxygenyl compound appears to be a very "clean" reagent for air-purification purposes. It is less corrosive than such complex fluorides as ClF_2SbF_6 , BrF_2SbF_6 , and BrF_2BiF_6 , and may be stored in glass or fluorinated plastic containers. Very little decomposition has been noted, for example, in samples that have been stored for 6–9 months in 'Pyrex' bulbs and 'Kel-F' test tubes. Some corrosion of 'Kel-F' has been observed, however, by products formed in the reaction with xenon—probably excited xenon, oxygen, or ozone species. The compound is decomposed by moisture; in treatment of wet gases, it must therefore be used in conjunction with a desiccant, such as silica gel or a molecular sieve.

The dioxygenyl compound can probably be used for such purposes as purifying radon-contaminated atmospheres in uranium mines; removing xenon isotopes from reactor and reprocessing plant off-gases; separating xenon from lighter noble gases; and analysing xenon and radon isotopes in air. In one of the methods which is being developed to reduce emissions from boiling-water reactors, large beds of charcoal are used to "hold up" radioisotopes^{2,3}. Waste gases are passed through the beds at ambient temperature, and the noble gases are adsorbed and retained long enough for short-lived isotope to decay. (Xenon isotopes may be held for 20 days and krypton isotopes for about 1 day.) Small beds of O_2SbF_6 can probably be substituted for the large (100 to 200 ton) beds of charcoal to remove xenon isotopes from the waste gases completely.

Further research may disclose oxidants that can be used to collect krypton as well as radon and xenon. Krypton forms a simple fluoride, KrF_2 , which is stable only at low temperatures, and a complex fluoride, $\text{KrF}_2 \cdot 2\text{SbF}_5$, which is stable at 25°C (refs. 21, 22). (The latter is probably an ionic compound, $\text{KrF}^+\text{Sb}_2\text{F}_{11}^-$, analogous to $\text{XeF}^+\text{Sb}_2\text{F}_{11}^-$.) By substituting cations of very great oxidizing power for the O_2^+ cation in O_2SbF_6 , it may be possible to prepare reagents that combine spontaneously with krypton to form this stable compound.

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Inverse Mass Dependence in Liquid Diffusion of Heavy Ions

AFTER the failure of early attempts to find isotope effects in liquid diffusion it was assumed¹ that these effects would be destroyed by two mutually antagonistic mechanisms. Firstly, a first approximation gas model resulted in higher speed for

than in gases. Recently⁵ liquid diffusion cascades have been used in the search for isotope effects in liquid diffusion. A normal gas-like isotope effect was found for the Li isotopes in polyethylene glycol (PEG) solution but the inverse effect occurred for the U isotopes. Only one experiment has shown the same mass dependence: Cini-Castagnoli *et al.*^{6,7} investigated the diffusion of argon in liquid nitrogen and found slower diffusion for ³⁷A than for ⁴⁰A. The difference was up to 10% but it was not emphasized and was apparently considered to be within the range of experimental error.

Here I report further confirmation of this inverse mass dependence for diffusion of uranyl ions in aqueous solution and in agar gel. A prismatic cascade⁸ 60 cm high, with a cross section 10 by 60 cm at the top, containing 59 barriers of nylon gauze, was filled with distilled water. Uranyl nitrate solution with density 1.194 was introduced at the top. A layer of glass powder on the top barrier prevented convection of this concentrated solution. After 21 days a sample was drawn from the bottom which showed a depletion of ²³⁵U against the blank. The result is given in Table 1. The simple process factor ϵ , which may be deduced from these results, has the same order of magnitude as previously⁶. Hydration of the uranyl ion does not suppress appreciably the isotope effect.

It was possible to improve the efficiency of the barriers by using cylinders of sintered polystyrene beads impregnated with agar gel or solid agar cylinders supported by stainless steel gauze. The diffusion proceeded in radial direction towards a central channel. Details of this method will be published elsewhere. Again a depletion of ²³⁵U occurred as shown in Table 1.

Table 1 Depletion of ²³⁵U in Solution after Passage through Barriers

System	Stage No.	Type	Cascades Material of barrier	Diffusion time	²³⁵ U/ ²³⁸ U
Uranyl nitrate in water	59	Prismatic	Nylon gauze	21 days	Sample: 0.007191 ± 0.000013* Blank: 0.007418 ± 0.000015*
Uranyl nitrate in agar gel	2	Cylinders 47 mm diameter	Agar gel in polystyrene matrix	3 days per stage	Sample: 0.007132 ± 0.000019* Blank: 0.007369 ± 0.000029*
Uranyl nitrate in agar gel	1	Cylinder 125 mm diameter	Agar gel	4 days	Sample: 0.007208 ± 0.000022 Blank: 0.007285 ± 0.000018

* Arithmetical mean from 2 runs.

the lighter isotopes; secondly, however, in liquids the lighter isotopes would meet with an increased backscatter and would have less persistence than the heavier isotopes. Thus the preference of the mass independent Stokes-Einstein equation appeared justified for a first approach to liquid diffusion coefficients. The slower diffusion of the lighter alkali and alkaline earth ions was attributed wholly to the larger diameters caused by hydration and not to mass effects. The contribution of hydration will be indeed substantial for the lighter ions such as Li and Na but this influence has never been proved quantitatively especially for the heavier ions such as Sr and Ba.

The hydrodynamic model has been questioned by Lindemann². He suggested a kinetic model and explained the slow motion and the high solvent transfer of the Li ion by momentum transfer.

The kinetic approach of Arnold³, however, did not take into account the persistence correction. Without this correction by Jeans⁴ the Stefan-Maxwell equation could not explain the weak composition dependence of the diffusion coefficients in gas mixtures. In the case of a few heavy molecules diffusing in an excess of light molecules the persistence correction results in a substantial increase in the diffusion coefficient against the uncorrected value.

Because of the higher collision number in liquids the effect of persistence should play a still more important role in liquids

I conclude that the mechanism of persistence and backscatter plays an even more prominent role in the diffusion of heavy solutes in light solvents than was assumed by Hevesy and Paneth¹. This mechanism not only compensates but overcompensates the features of the gas model and results in inverse mass dependence.

I thank Dr N. C. Fenner for carrying out the mass spectrometric analysis.

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Artificial Semiconductors

IN 1968 while spending a sabbatical period at the Allen Clark Research Centre I worked on an idea for making artificial semiconductors and did a few preliminary experiments. It seems worth recording these because, due to the interest of colleagues, we are about to continue the work; and a comparable scheme has been proposed by distinguished scientists at IBM¹.

The basic idea is to make a multilayered structure of alternate thin films of metal and insulator. The resulting potential diagram (Fig. 1) is similar to the Kronig-Penney² representation of a crystalline solid. In a solid, of course, the potential barrier is the effort an electron has to make to get from one atom to the next and its width is entirely governed by the crystal lattice spacing, a very inflexible parameter. Because nearly all lattice spacings are between 3 and 5 Å, it follows, from solving the Schrödinger equation with barriers of this periodicity, that the energy spread of a band is a few electron volts. The limits of the band occur when the periodic distance, $(a+b)$ in Fig. 1, is an integral number of half de Broglie wavelengths for an electron.

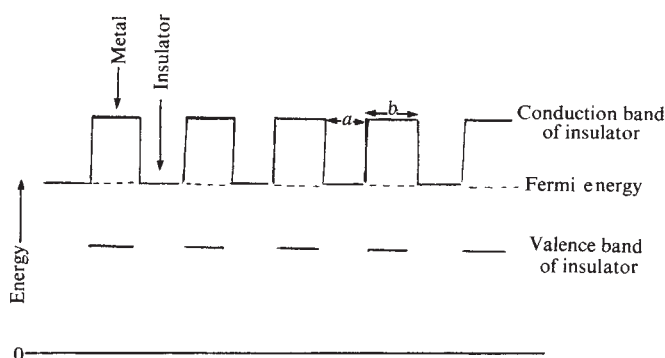


Fig. 1 Potential diagram of a multilayered structure.

If one applies this model to dimensions that are just practicable for an artificial semiconductor, say, sputtered layers of 20 to 30 Å, a great difference occurs. The solution of the Kronig-Penney problem simplifies to an approximate equation

$$\alpha_n a = K \pm n\pi$$

where $\alpha_n = (2mE_n)^{1/2}/\hbar$, E_n is the energy of the n th allowed level, a is the thickness of the metal and K is a constant for a particular structure.

The values of α_n represent allowed energy levels. The allowed band is very narrow, a physical consequence of the wide barrier which holds the electrons to the metal layers. Consequently, once the values of the Fermi level of the metal and the energy gap of the insulator are known it is easy to calculate the energy gap corresponding to, say, the transition from $n=0$ to $n=1$. For a Au-SiO₂ multilayer with $a=30$ Å this comes to about 1.0 eV.

In the experiments I was associated with in 1968 we used an r.f. sputtering set with a rotating electrode, one half SiO₂ and the other half Au. Each side could be made to sputter on to substrates separately. The sputtering rates were determined in the usual way by measuring long exposures, then the times were scaled down so that a thin layer of each material was obtained. Typical runs were made in which about twenty layers each of Au and SiO₂ were sputtered on to glass or 'Irtran' substrates.

Of course a 30 Å layer even by r.f. sputtering must have many voids and islands. Electrical resistance measurements

on a forty-four layer structure, showed an ohmic characteristic with a resistance of only $\sim 10^{-2} \Omega$. This was presumably the path of lowest resistance through the layers and may well have been shunting some more interesting characteristics. A more promising diagnostic method is infrared absorption. At the infrared wavelength corresponding to an energy gap electrons should be excited across it, with the corresponding absorption of radiation. Two runs of multilayered structure were therefore sputtered on to an 'Irtran' substrate which has a good transmission characteristic. Infrared from a hot source and a grating monochromator was split into two equal beams one of which was transmitted through the specimen and the other through a blank 'Irtran' substrate. The two beams were detected and the difference signal fed on to a pen recorder. The pen recorder was driven by the same motor that operated the monochromator. The first specimen tested was a forty-four layer structure with nominal 30 Å Au separated by nominal 20 Å SiO₂. An absorption edge well above noise level was found at 9.5 μm with smaller edges at 7.9 μm , 4.3 μm and 3.05 μm . The second specimen, which had only eighteen layers, showed less marked effects. The large discrepancy between the calculated value of 1.0 eV and the measured value of 0.13 eV for the principal absorption edge indicates either that the simple theory is inadequate or perhaps that it is wrong to take a value for the bulk Fermi energy of gold (5.6 eV) in considering thin layers. The value of the calculated energy gap is sensitive to this value, decreasing to 0.04 eV if the Fermi energy is zero.

In spite of this limitation of theory the prospect of achieving man-made semiconductors is an exciting one. These structures are difficult to make, so it is too early to be definite about their application. The idea of tailoring a photoconductor for specific narrow band infrared detection is, however, attractive. It is also likely that negative resistance characteristics could be created if a higher uniformity could be achieved. Further experimental and theoretical work is needed to explore these possibilities.

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BIOLOGICAL SCIENCES

Role of ATP in DNA Replication

THE bacterial chromosome replicates in a semiconservative manner¹. This process may occur at the cell membrane^{2,3}. Efforts to study replication by means of purified DNA polymerases have failed to elucidate the mechanism of the *in vivo* process. Therefore, recent attempts have been made toward isolation of membrane-containing systems which more nearly approximate the properties of *in vivo* replication. Two different procedures have been used. One relies on hydrolysis of the cell wall by lysozyme, followed by gentle disruption of the spheroplast membrane so as to avoid shearing the replication apparatus^{4,5}. In a different approach, cells have been made permeable to nucleotides and other small molecules by treatment with toluene⁶⁻⁸ or ether⁹. This permits incorporation studies with little alteration of the cell interior. Both of these procedures yield preparations that replicate bacterial or phage

DNA semiconservatively in the presence of ATP, as measured by pycnographic analysis of the product⁹⁻¹¹. In the absence of ATP⁶, synthesis appears to be of the repair type¹². In these systems, ATP does not merely protect the deoxynucleoside triphosphate pool^{9,13} or stimulate the rec B, C nuclease⁶.

These observations suggest that ATP has some specific role in the replication process. To examine the nature of this role, a membrane preparation has been derived from a wild type strain of *Escherichia coli*, W3110, which exhibits many of the properties of the *in vitro* replicating systems described above. We present evidence that in this system the presence of ATP suppresses the utilization of deoxynucleoside triphosphates for repair synthesis and diverts them to replicative synthesis. In these conditions the incorporation of precursor shows unusual kinetics.

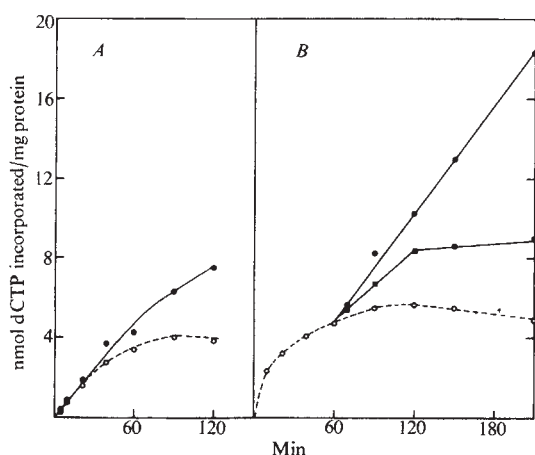


Fig. 1 Kinetics of DNA synthesis by the membrane preparation. *E. coli* W3110 was grown in T-broth¹⁴ to a density of about 5×10^8 cells/ml., washed and resuspended in 0.05 volumes of 0.05 M Tris buffer, pH 7.4, containing 20% sucrose. The suspension was treated with 0.005 M EDTA and 50 μ g/ml. of egg white lysozyme for 15 min at 30°C. The resulting spheroplasts were sedimented at 10,000g in the Sorvall centrifuge for 10 min. The pellet was subjected to two cycles of quick freezing and thawing. The lysed spheroplasts were resuspended in 0.05 volumes of 0.05 M Tris buffer, pH 7.4, containing 0.01 M $MgCl_2$ (Mg-buffer) and centrifuged at 1,500g for 5 min. The supernatant was again centrifuged at 15,000g for 15 min. The pellet was washed once with the same volume of Mg-buffer and resuspended in 0.02 volumes of that buffer (membrane). Unless otherwise stated, 150 to 200 μ g of membrane protein was used per ml. of reaction mixture. **A**, The reaction was carried out at 37°C in a mixture containing 55 μ M each of dATP, dGTP, TTP and ³H-dCTP (5 μ Ci/ml.), 8 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.8, and 0.33 mM ATP where indicated. Aliquots of 0.1 ml. were withdrawn and dried on 2.3 cm Whatman No. 3 filter paper disks, which were washed several times with 0.3 M trichloroacetic acid, once with acetone and then dried. The dried disks were transferred to vials containing 5 ml. of toluene-based scintillation fluid and counted in a scintillation spectrometer. **B**, ATP (0.33 mM) or deoxynucleoside triphosphates (25 μ M each) including ³H-dCTP (2.3 μ Ci/ml.) were added to different aliquots of a minus-ATP reaction mixture at 60 min. ●, Plus ATP; ○, minus ATP; ■, plus deoxynucleoside triphosphates.

The incorporation of ³H-dCTP into DNA by these membrane preparations continued for 40 to 60 min, and then ceased (Fig. 1A). In the presence of ATP the reaction continued for at least another hour. If ATP was added 60 min after the start of a reaction, when incorporation of ³H-dCTP had ceased, incorporation resumed immediately and continued at a linear rate for 2 h (Fig. 1B).

Sustained DNA synthesis in the presence of ATP may result from the regeneration of deoxynucleoside triphosphates that are hydrolysed during the reaction. To examine this possibility, aliquots were taken at several times throughout the course of a reaction and analysed by thin-layer chromatography

for the fate of ³H-dCTP. The results are shown in Fig. 2A. By 60 min only 14 nmol of the original 50 nmol of triphosphate remained in the reaction lacking ATP, whereas 25 nmol of triphosphate was present in the reaction containing ATP. However, when an additional 25 nmol of each of the four triphosphates was added to a reaction mixture lacking ATP at 60 min to replenish the substrates, incorporation resumed, but at only 60% of the rate attained when ATP was added and for only 60 min (Fig. 1B). Furthermore, the addition of 50 nmol of the four triphosphates did not increase the rate of incorporation beyond that observed with 25 nmol. Thus, although ATP did protect the deoxynucleoside triphosphate levels to some extent, it may have an additional function that cannot be replaced by the deoxynucleotides.

To examine this possibility further, the effect of the α,β -methylene analogue of ATP was studied. When added to a reaction mixture containing ATP, the analogue did not prevent the ATP-induced protection of ³H-dCTP (Fig. 2A). However, it completely prevented the sustained incorporation of ³H-dCTP produced by ATP (Fig. 2B). Therefore, the analogue seems to separate the requirement of ATP for triphosphate regeneration from some additional role in DNA synthesis. We shall now show that ATP has a qualitative effect on DNA synthesis, beyond its ability to maintain adequate levels of the deoxynucleoside triphosphates.

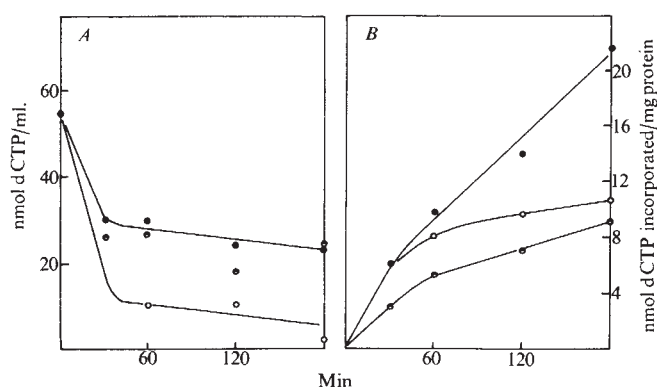


Fig. 2 Effects of ATP and its α,β -methylene analogue on deoxynucleoside triphosphate level and DNA synthesis. **A**, Degradation of ³H-dCTP. The incubation mixture was the same as described in the legend to Fig. 1 except that ATP (0.33 mM) and α,β -methylene ATP (1.0 mM) were added as indicated below. Aliquots of 0.05 ml. were withdrawn and deproteinized by treatment with 0.3 M perchloric acid after adding 100 μ g of bovine serum as carrier. The precipitate was removed by centrifugation. The supernatant was neutralized by KOH and analysed by PEI-cellulose thin-layer chromatography for nucleotides. The chromatogram was developed for 2 h with 0.1 M acetic acid-1.2 M LiCl. **B**, Incorporation of ³H-dCTP. The incubation mixture was the same as in **A**. Incorporation of radioactivity into DNA was measured as described in Fig. 1. ●, Plus ATP; ○, minus ATP; ■, plus ATP and α,β -methylene-ATP.

Sulphydryl compounds inhibit replicative synthesis but not repair synthesis in permeabilized cells and membrane preparations that require ATP for semiconservative DNA replication⁵⁻⁷. In our system (Table 1), N-ethylmaleimide (NEM) slightly stimulated ³H-dCTP incorporation in the absence of ATP, but inhibited incorporation by about 65% in the presence of ATP. Since the initial rate of DNA synthesis in the presence and absence of ATP was the same (Fig. 1A), these results indicate that ATP induces the NEM-sensitive replicative type of DNA synthesis and simultaneously suppresses the NEM-insensitive repair type of synthesis in these preparations.

To characterize further the nature of the reaction, the product synthesized in a reaction mixture in which dBrUTP replaced

TTP was analysed pycnographically. It was found that more than half of the product showed a hybrid density when ATP was included in the reaction (Fig. 3). Absence of ATP resulted in the synthesis of light DNA only. These results show that ATP is essential for semiconservative replication in this system and that it can partially suppress the repair type of DNA synthesis.

ATP may act at more than one step in the process of DNA replication. We examined the assumption that one role of ATP is to alter some component required for DNA replication, either substrate, primer or enzyme, which is common to both the repair and replicative systems. This process might be observed as a brief interval during which the labelled deoxynucleoside triphosphate cannot be utilized. Fig. 4 shows that when ATP and ^3H -dCTP were added to a membrane preparation after 40 min of DNA synthesis in the absence of ATP, label was not incorporated into acid precipitable material for about 100 s. By contrast, in the control experiment where no ATP was added with ^3H -dCTP, no lag of incorporation could be detected. A shorter lag of 20 to 30 s was observed when the ATP and ^3H -dCTP were added at the very start of synthesis. The longer lag at 40 min may result from the much lower rate of repair synthesis occurring at that time (Fig. 1). In reaction mixtures containing purified DNA polymerase I in place of the

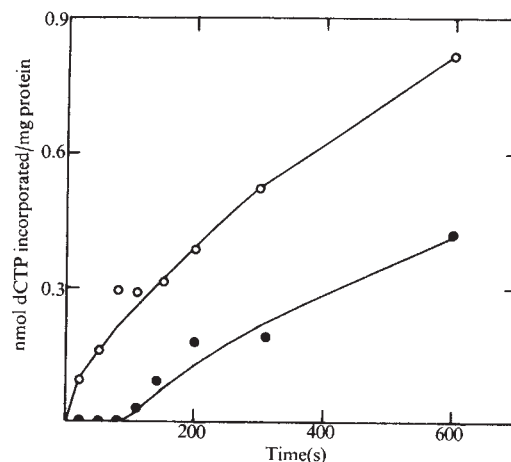


Fig. 4 Effect of ATP on the incorporation of ^3H -dCTP. A reaction mixture like that described in the legend to Fig. 1, containing four deoxynucleoside triphosphates but no ATP or ^3H -dCTP, was incubated at 37°C for 40 min. ATP (0.33 mM) and ^3H -dCTP (20 $\mu\text{Ci}/\text{ml}$.) were then added. Aliquots of 0.10 ml. were withdrawn at indicated time intervals and prepared for counting. ●, ATP added with ^3H -dCTP; ○, ^3H -dCTP added alone (no ATP).

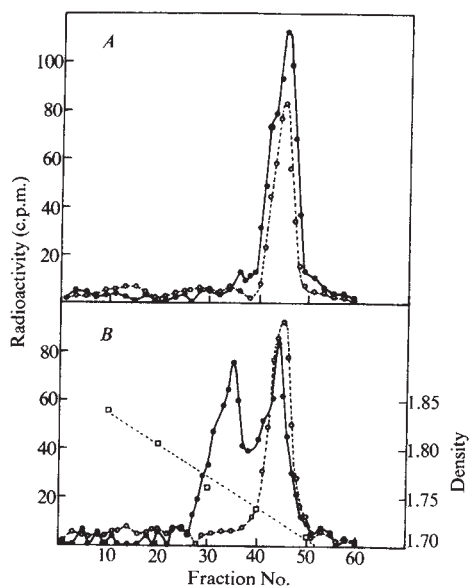


Fig. 3 Pycnographic analysis of the product. The incubation was carried out at 37°C for 20 min in a reaction mixture like that described in the legend to Fig. 1 except that TTP was replaced by dBrUTP, either (A) in the absence of ATP, or (B) in the presence of ATP. Aliquots of 0.3 ml. of the reaction mixture were mixed with ^{32}P -labelled *E. coli* DNA and 0.10 ml. of 1.5% 'Sarkosyl'-0.05 M Tris-HCl, pH 7.3-0.01 M EDTA. The clear mixture was then sheared according to the method of Burger¹⁵, mixed with 4.5 ml. of CsCl (density $1.808\text{ g}/\text{cm}^3$) and centrifuged in the Spinco rotor SW 39 at 36,000 r.p.m. for 64 h at 22°C . Fractions of two drops were collected directly on filter paper disks. The density of selected fractions was determined refractometrically. The paper disks were treated and counted as described in the legend to Fig. 1. ●, Product; ○, marker ^{32}P -labelled *E. coli* DNA; □, density.

membrane preparation, ATP induced no lag of incorporation, showing that the presence of ATP did not directly prevent utilization of deoxynucleoside triphosphates.

In an effort to determine the basis for this lag of incorporation of ^3H -dCTP, we tested for the presence of this nucleotide 150 s after its addition to a reaction mixture like that shown in Fig. 4. The mixture was analysed by DEAE-'Sephadex' chromatography. Fig. 5A shows that more than 60% of the

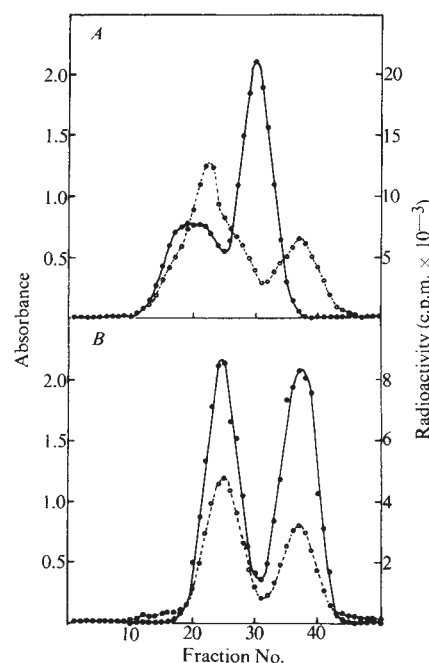


Fig. 5 Analysis of the reaction mixture by column chromatography. The incubation was carried out as described in the legend to Fig. 4, but with 400 μg of membrane protein per ml. of reaction mixture. After addition of ATP (0.33 mM) and ^3H -dCTP (100 $\mu\text{Ci}/\text{ml}$.) the mixture was incubated for 150 s and then chilled. The material was mixed with dCTP and dCDP and adsorbed to a column of DEAE-'Sephadex A-50'. The adsorbed nucleotides were eluted and rechromatographed with a linear gradient from 0 to 0.25 M KCl in 0.05 M Tris-HCl, pH 7.9. Fractions of 1.2 ml. were collected. A, Plus ATP. B, Minus ATP. ●, Radioactivity; ○, absorbance at 260 nm.

Table 1 Effect of NEM on DNA Synthesis by Membrane Preparations

Reaction mixture	nmol ^3H -dCTP/mg protein/20 min
Complete (+ ATP)	1.944
Complete+NEM (20 mM)	0.667
Complete-ATP	1.888
Complete-ATP+NEM (20 mM)	2.611

radioactivity eluted from the column at a position intermediate between dCDP and dCTP with little material at the position of the original triphosphate. In contrast, no material was found at this elution position when the reaction was carried out in the absence of ATP (Fig. 5B). The large amount of dCDP in the control reaction probably resulted from the high concentration of membrane protein that was used in these experiments. Examination of the altered material by sucrose gradient sedimentation showed that it did not sediment faster than pure ^3H -dCTP. This indicates that its altered chromatographic position did not result from binding to macromolecular components of the reaction mixture. It is not yet clear whether the other three deoxynucleoside triphosphates experience a similar alteration.

Thus, the membrane preparation from *E. coli* W3110 exhibits two of the types of DNA synthesis that are observed *in vivo*—repair and replication. ATP seems to play a key role in the control of these reactions. In the absence of ATP, synthesis occurred only by the repair mechanism. This process was insensitive to NEM and resulted in an imperceptible change in density of the template DNA when density-labelled precursor was present in the reaction mixture. The results indicate that ATP suppressed this type of synthesis. In the presence of ATP, incorporation of label occurred at the same rate but was inhibited by NEM by more than 65%, and more than half of the product showed a hybrid density after synthesis in the presence of density-labelled precursor. This indicated that more than half of the synthesis was due to extensive semi-conservative replication.

ATP may regulate the relative activity of these alternative mechanisms of DNA synthesis by altering some component involved in the replication process. This would account for the 100 s lag before incorporation of labelled deoxynucleoside triphosphate into DNA when ATP was present, and immediate incorporation when ATP was absent.

It is likely that the synthesis of a short polyribonucleotide primer is essential for the initiation of DNA replication. Isolation of replicating DNA covalently linked to small polyribonucleotide fragments¹⁶, and the requirement of RNA synthesis before the onset of DNA replication in several systems^{17–19}, support this idea. Synthesis of such primer polyribonucleotide could block the site for repair synthesis resulting in the transient halt of both synthetic activities. In our system ATP is the most efficient of the ribonucleoside triphosphates for stimulating DNA synthesis, and a mixture of all four ribonucleoside triphosphates did not stimulate the formation of hybrid DNA more than did ATP alone. This suggests that the putative polyribonucleotide primer is predominantly polyadenylic acid.

The alternative explanation for the role of ATP is that it modifies the deoxynucleoside triphosphates and that this explains the incorporation lag shown in Fig. 4. It remains to be shown whether this modification of dCTP is an important or trivial aspect of the role of ATP in DNA replication.

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Specificity and Molecular Features of an Insect Attractant in a *Drosophila* Mutant

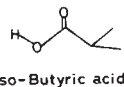
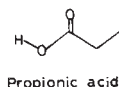
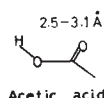
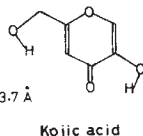
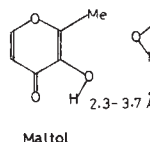
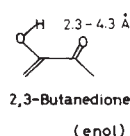
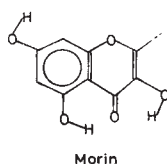
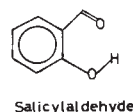
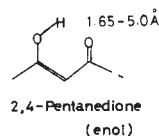
SEVERAL attractants and repellents which are highly specific for a given species or sex have been identified in the last decade. Although much effort has been concentrated on demonstrating the molecular basis of odour perception in insects, the mechanisms involved are still far from clear¹. As the complicated network of cellular function in chemotaxis has been successfully analysed in bacteria, using a mutation which indicates alteration of one element involved at a time, a similar approach is expected to be fruitful in studies of the olfactory system^{2,3}. *Drosophila* is the insect of choice for such studies because of its large stock of marker genes and crossing-over suppressors which are essential for the isolation of induced olfactory mutants. Recent success in the isolation of behavioural mutants of *Drosophila* has thrown light on this subject⁴. I shall describe here the properties of an olfactory mutant of *Drosophila melanogaster* and the molecular characteristics of the odours to which the mutant responded specifically.

Using a potent mutagen for *Drosophila*, ethyl methane sulphonate (EMS), an olfactory mutant (*HPB-1*), showing considerable attractiveness to 4-(*o*-hydroxyphenyl)-3-buten-2-one (HPBO), was isolated from the acetic acid-attractive line (*AA75*) which was originally established from the *Oregon R* wild strain. The details of the isolation of the mutant and its genetic properties will be described elsewhere. The mutation probably involves a dominant gene in the second chromosome.

Behavioural changes in the mutant flies in response to various odours and water were examined using an olfactometer, and compared with those of the parent flies. Flies aged 0–36 h cultured on a corn-yeast medium were starved for 28 h at 25° C. Two hundred and fifty millilitres of freshly prepared odour solution and the same amount of distilled water were each kept in a gas washing bottle at 20° C. Four hundred millilitres of two testing vapours, approximately equal to the capacity of each olfactometer channel, was passed at a rate of 100 ml. min⁻¹ for 4 min through two arms of the olfactometer. Tests were done at 25° C in the dark, at a flow rate of 2 ml. min⁻¹ for 1 h. The flies trapped in each trapping bulb were cooled and counted. The responses of the mutant flies to given odours were evaluated by determining the ratio of the number of flies trapped in the odour side of the olfactometer to the total number trapped in the two bulbs (R_m), and comparing it with that of parent flies (R_p).

Of sixty-four odours examined, the mutant was specifically attracted by twelve chemicals which were repellent to the parent

Specific



Non-specific

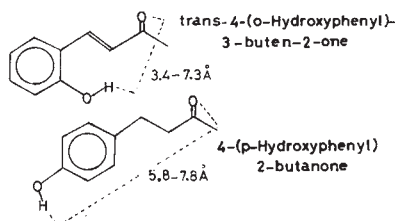
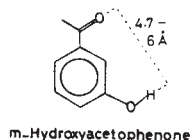


Fig. 1 Comparison of the molecular structures of specific odours with those of non-specific ones. Distances between =O atom and -OH proton were estimated by the chemical model, W. Büchi Glasapparatefabrik Flawil.

strain (A475). All the other fifty-two odours were non-specific. (Odours are defined as specific where $R_m - R_p > 15\%$.) Table 1 shows that these twelve specific odours, included a variety of chemicals: α and β -diketones (2,3-butanedione and 2,4-pentanedione), aliphatic monoketones (2-propanone, 3-heptanone and 5-nonanone), γ -pyrones (kojic acid and maltol), a flavon (morin), an aldehyde (salicylaldehyde), monocarboxylic acids (acetic acid, propionic acid and iso-butyric acid). The results obtained so far indicate that certain functional groups such as methyl, methoxyl and phenyl do not play an important role in producing the present specificity; nor do molecular size or shape seem to be relevant. Hydroxyl, carbonyl, aldehyde and carboxylic groups, on the other hand, appear to effect the specificity. The structure of each of the seven specific odours, maltol, kojic acid, morin, salicylaldehyde and three carboxylic acids, includes a proton acceptor (=O) and a proton donor (-OH), the minimal distances between which are 1.65 to 2.5 Å. Furthermore, in the gas phase 2,4-pentanedione apparently assumes the enol form (almost 90%) enolization⁵, forming a structure similar to salicylaldehyde and morin. The α -diketone, 2,3-butanedione, contains about 0.6% of the enol form in the absence of solvent⁶, forming a structure similar to maltol and kojic acid. So these nine chemicals contain any one of the following three structures (Fig. 1): (1) the 2,4-pentanedione type, including salicylaldehyde and morin where the distance between the =O atom and -OH proton (A-D distance) ranges from 1.65 to 5.0 Å, giving an average value ($\bar{AD}$) of about 3.3 Å; (2) the 2,3-butanedione type including kojic acid, maltol and morin where the A-D distance is 2.3-3.7 Å for γ -pyrones and flavon ($\bar{AD}$: 3.0 Å) and 2.3 to 4.3 Å for 2,3-butanedione ($\bar{AD}$: 3.3 Å); and (3) the acetic acid type, including three carboxylic acids the A-D distance of which ranges from 2.5 to 3.1 Å ($\bar{AD}$: 2.8 Å).

My assumption that the "bifunctional unit" plays a part in the present specificity is supported by the following facts: (1) None of ten alcohols and phenols such as ethanol, pyrocatechol, o-hydroxybenzyl alcohol, 4-methyl-2-pentanol and

nerol showed specificity. (2) All six chemicals in which the average A-D distance ($\bar{AD}$) ranges from 5.3 Å (dimedone, enol⁷) to 6.9 Å (p-hydroxypropiophenone) were entirely non-specific. (3) Of six chemicals having two proton acceptors, the average distance between the two oxygen atoms of which ranged from 3.1 Å (2,5-hexanedione and dimethylsuccinate) to 5.2 Å (dimethylfumarate), none was specific for the mutant.

On the other hand, in the three monoketones (2-propanone, 3-heptanone and 5-nonanone), which are in the specific odour group, absorption was shown by infrared spectrometry to be attributable to the hydroxyl group. This implies that the present specificity is not a consequence of impurities having a hydroxyl group, but of the chemicals themselves. One explanation based on the "bifunctional unit model" is, however, possible if it is assumed that there are two molecules of enol and keto forms which fulfil the role of proton acceptor and donor. But knowledge of the keto-enol equilibria of these chemicals in gas phase is vague. The last mentioned chemical, 4-(o-hydroxyphenyl)-3-buten-2-one (Eastman Organic Chemicals, USA), which was the first to be described as a specific odour and showed the highest degree of specificity among chemicals examined (Table 1), contained a *trans* isomer as its chief constituent and an unknown trace; both of them, however, turned out to be non-specific. Although the *cis* isomer certainly has the characteristic structure where the A-D distance is 0.8-2.6 Å (neglecting the interatom repulsion), it is not yet conclusively known whether the present specificity is attributable to the *cis* isomer, as the latter has not been isolated. In addition a nuclear magnetic resonance experiment (NMR) gives no signal which can be attributed to the *cis* configuration. If the "bifunctional unit" is a common feature of the odour molecule of different specific compounds it follows that there may be a particular receptor site, also constituting a bifunctional unit, of a similar nature and that the interaction between the odour molecule and the receptor site is due to two simultaneous hydrogen bonds. Several compounds with specific odours, such as 2,4-pentanedione, morin, salicylaldehyde and acetic acid, are well-known chelating agents for heavy metals. It has been suggested that certain heavy metals could play a part in olfactory perception of the house fly, as injection of the metal chelating agent 'Nitroso-R' causes changes in response to n-butanol⁸.

Table 1 Responses of Mutant and Parent Flies to Odours

Odour	Concentration (M)	Response (%) [*]		Difference (%)
		Parent (R_p)	Mutant (R_m)	
2,4-Pentanedione	10^{-1}	35	58	23
Salicylaldehyde	10^{-3}	40	63	23
Morin	2×10^{-4}	43	61	18
Maltol	10^{-3}	39	60	21
Kojic acid	10^{-3}	35	55	20
2,3-Butanedione	10^{-2}	39	62	23
Acetic acid	10^{-3}	27	53	26
Propionic acid	10^{-1}	39	69	30
iso-Butyric acid	10^{-2}	42	59	17
2-Propanone	4×10^{-1}	52	79	27
3-Heptanone	10^{-5}	34	57	23
5-Nonanone	10^{-4}	21	53	32
4-(o-Hydroxyphenyl)-3-buten-2-one	2×10^{-4}	33	71	38
Diacetone alcohol	10^{-1}	37	37	0
Phthiocol	10^{-4}	86	82	-4
3-Hydroxyflavon	10^{-5}	78	73	-5
Tropolone	10^{-1}	44	47	3
m-Hydroxyacetophenone	2×10^{-3}	73	68	-5
4-(p-Hydroxyphenyl)-2-butanone	5×10^{-4}	76	70	-6
trans-4-(o-Hydroxyphenyl)-3-buten-2-one	2×10^{-4}	58	54	-4

^{*} Represented as the ratio of the number of flies trapped in the odour side of olfactometer to the total number of flies trapped in the odour and water side. No. of tests, 113; No. of flies trapped, about 28,500.

A variety of chemicals which are definitely non-specific have the bifunctional unit. These include the 2,4-pentanedione type: *o*-hydroxyacetophenone and *o*-hydroxypropiphenone; the 2,3-butanedione type: 3-hydroxyflavon and phthiocol; the acetic acid type: fumaric acid and several carboxylic acids. Other non-specific odours, such as tropolone, purpurogallin, diacetone alcohol and acetoin, may belong to a similar category, as they have a feature closely resembling the bifunctional unit. It seems then that there are still unknown features which have certain functions in the interaction between the odour molecule and the receptor site.

A characteristic feature of the present mutation is that the mutant was strongly attracted by chemicals which repelled the parent strain. The behaviour of the mutant might be explained if it contains a missense mutation producing a modification or defect in a receptor site which interacts with the repellents or in the mutant's nervous system. The fact that the mutant is strongly attracted by chemicals which repel the parent flies implies that there is at least a different site, and that the neural excitation thus produced leads after integration to an orientated movement towards the odour source. This indicates that odours act on different sites rather than on any particular one. Thus, the results obtained so far suggest that the use of olfactory mutants could lead to the identification of receptor sites and to the isolation of substances involved in the olfactory system. Finally, the use of such mutants should be of help in analysing the complicated network of the olfactory system.

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Collection, Motility and Storage of Spermatozoa from the African Elephant *Loxodonta africana*

NATURAL breeding of elephants in zoos is uncommon because bulls have proved dangerous to handle and are not usually kept. The collection and storage of elephant semen and artificial insemination are, therefore, being investigated by the Zoological Society of London as part of a research project on the preservation of mammalian spermatozoa¹. Problems of semen collection² were simplified by taking advantage of the culling programme in progress at the Kruger National Park, South Africa.

Mature bull elephants, 15 to 50 yr old, were immobilized by shooting with darts that injected 'Scoline' (Glaxo Laboratories Ltd) on impact, then bled from the jugular vein and eviscerated.

The reproductive tract was removed within 15 min of death and the excurrent duct of the testis dissected. Spermatozoa were removed by flushing the duct posteriorly. There were many more in the distal region and they showed such structural signs of maturity as a condensed acrosome and a distally-situated protoplasmic droplet. These characteristics were absent in spermatozoa recovered from the proximal portion of the duct.

The report of Short *et al.*³ that spermatozoa removed from the distal portion of the duct were immotile was confirmed; only one sample examined before dilution showed any signs of activity and in this only 15% of spermatozoa showed weak flagellation. Various methods of inducing motility in these spermatozoa were tested. Subsamples (0.03 ml.) of effluent flushed from the duct with air were diluted into 15 ml. tubes containing 2.0 ml. of buffered saline (5 mM sodium phosphate and 149 mM sodium chloride, pH 6.8) alone, or including 5 mM potassium chloride or 10 mM fructose or both substances. Some samples were examined with a field microscope to confirm that the spermatozoa were motile immediately after dilution. Tubes were stoppered and transferred in a vacuum flask, at 30 to 35°C, to a temporary laboratory. After storage for 2 to 3 h they were examined microscopically at ambient temperature (20 to 25°C) and motility was observed in all samples. Table 1 shows mean scores of the rate of progressive motility⁴ (range 0 to 4) and percentage of motile spermatozoa. The addition to the diluent of potassium, fructose, or both increased the proportion of motile spermatozoa ($P < 0.05$).

Table 1 Mean* Scores of Viability of Elephant Spermatozoa in Various Diluents and after Incubation for 3 h at 30°C

Diluent	Motility score	Motile spermatozoa (%)
Buffered saline	1.8	30
Buffered saline + KCl	2.3	40
Buffered saline + fructose	2.3	40
Buffered saline + KCl + fructose	3.3	50
Standard error of means †	0.60	4.7

* Results are means of samples from three elephants.

† From residual mean square in analyses of variance.

Several experiments were also carried out to determine a suitable method of freezing spermatozoa. Spermatozoa were flushed from the duct with a diluent which consisted of a 1:1 mixture of egg yolk and buffered citrate. The latter contained 20 mM sodium phosphate and 80 mM sodium citrate (pH 6.8). The flushings were then further diluted with the egg yolk citrate to achieve a rate of approximately four-fold (approximately 1×10^9 spermatozoa ml.⁻¹) and cooled to 5°C for 2 to 3 h. They were then diluted 1:1 with the buffered citrate containing a protective agent (dimethyl sulphoxide (DMSO) or glycerol), equilibrated for at least 0.5 h and then frozen in 0.5 ml. Cassou⁵ straws over liquid nitrogen (-196°C). Frozen samples were stored for at least 24 h before thawing. The efficacy of the two protective agents at final concentrations of 2, 4, 8 and 16% v/v was compared in a 2×4 factorially designed experiment, replicated using spermatozoa from four elephants. As judged by scores of progressive motility, the percentages of motile spermatozoa and unstained spermatozoa in eosin-nigrosin smears⁶, DMSO was more effective than glycerol and the best concentration of DMSO seemed to be 8%. Egg yolk-citrate diluent containing DMSO was better than egg yolk-glucose diluents⁷ containing glycerol or DMSO; a combination of 7% DMSO and 1% glycerol in egg yolk-citrate was even better than the diluent containing 8% DMSO. The combination was used to prepare a bank of deep-frozen spermatozoa; Table 2 gives details of the quality of the elephant semen samples stored in this bank after transport to, and storage for, 3 weeks at the Wellcome Institute in London.

Attempts were also made to collect semen from live elephants following electrical stimulation. On two separate occasions, a bull elephant was immobilized with 'Immobilon' (Reckitt and Colman, Hull) and stimulated with a rectal probe using a variable output square wave stimulator powered by a 12 V battery. The probe was 66 cm long and 13 cm in diameter and consisted of four sets of electrical contacts, each set comprising four brass plates having surface dimensions of 2×2.5 cm. The four plates in each set were equally spaced around the circumference of the probe, which was wired so that a ring stimulus of bandwidth of 2.5 cm could be obtained in any one of four positions by bridging adjacent quadrants. The first position was 10 cm below the apex of the probe and positions 2, 3 and 4 were situated posteriorly and separated by spaces of 2 cm. The positions for optimal stimulation were found to be 1 and 2, when the probe was fully inserted into the rectum, using an output of 6 to 10 V, a pulse-frequency of 30 Hz and a duration of 12 ms. An erection occurred in the first elephant and, within 20 min, 80 ml. of a translucent viscous liquid had been collected which did not contain spermatozoa; the battery was suspected. A new battery was substituted and 260 ml. of a fluid of similar appearance was emitted from the second bull in 15 min; more could probably have been collected had the stimulus been continued. The fluid contained 4.8×10^6 spermatozoa ml^{-1} ; the motility score was 3.5 (range 0 to 4, ref. 4) and 60% of the spermatozoa were motile.

Table 2 Characteristics of Deep-frozen Elephant Spermatozoa Immediately after Thawing and Viability after Incubation for 1 and 2 h at 30° C

Elephant No.	Scores of viability examined						
	Immediately after thawing			After incubation for			
	Motility	% motile	% un-stained*	1 h Motility	1 h % motile	2 h Motility	2 h % motile
931	3.0	50	85	2.5	50	2.5	40
942	3.0	50	88	3.0	50	2.0	35
945	3.5	60	90	2.5	30	3.0	50
915	1.0	10	68	1.0	10	0.5	10
944	3.0	30	95	3.0	30	1.5	15
907	3.5	60	94	3.0	30	2.5	40
943	3.5	50	93	1.0	30	2.0	30
Mean	2.93	44.3	87.6	2.3	32.9	2.0	31.4
Standard error	0.34	6.3	3.5	0.34	5.2	0.31	5.4

* Percentage unstained (alive) in an eosin-nigrosin smear.

I suggest that elephant spermatozoa are immotile before ejaculation and that dilution can induce motility. As judged by present methods of assessing semen quality, it seems that, with one exception (see Table 2), elephant spermatozoa survived freezing and thawing as well as the deep-frozen cattle semen used at present for artificial insemination. The results of electro-ejaculation are encouraging and will provide a basis for further studies, particularly of the Asiatic elephant the continued existence of which in the wild is in question.

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Paradoxical Effect of Anti-thymocyte Serum on the Thymus

THE suppressive effects of anti-thymocyte serum (ATS) on cell-mediated immune responses¹⁻⁴ have been attributed to the selective depletion of recirculating T lymphocytes⁵. In this view, lymphoid tissues such as thymus which do not contain significant numbers of itinerant, blood-borne lymphocytes would be spared from the debilitating effects of ATS. The observation that the rate of recovery from the effects of ATS is substantially accelerated in the presence of an intact thymus^{6,7} is consistent with this idea. That the thymus is spared is also supported by studies of the distribution of labelled ATS immunoglobulin (IgG), which indicate that antibody penetrates the thymus very poorly⁸. These considerations do not, however, directly bear on questions concerning possible alteration in immunological activity of thymus cells after the peripheral administration of ATS. The following experiments demonstrate that graft-vs-host (GVH) activity produced by thymus cells is substantially increased for several weeks after the peripheral administration of a single dose of ATS. As this effect was not produced by normal rabbit serum and was removed by absorption of ATS with thymocytes, it seems to represent a specific immunological consequence of administration of ATS.

Table 1 Effect of Administration of ATS on GVH Reactivity of Splenic and Thymus Cells

Days after ATS	Cell No./thymus ($\times 10^{-6}$)	Spleen index thymus (5×10^6)	Spleen index spleen (5×10^6)
0	105 $\pm$ 6	1.13 $\pm$ 0.06	2.49 $\pm$ 0.06
1	96 $\pm$ 14	0.99 $\pm$ 0.03	1.16 $\pm$ 0.12
2	98 $\pm$ 10	2.37 $\pm$ 0.18	1.06 $\pm$ 0.08
3	101 $\pm$ 9	2.45 $\pm$ 0.14	ND
7	ND	2.08 $\pm$ 0.20	1.16 $\pm$ 0.11
14	100 $\pm$ 6	2.00 $\pm$ 0.16	1.48 $\pm$ 0.11
21	101 $\pm$ 8	1.50 $\pm$ 0.11	
28		1.34 $\pm$ 0.14	1.98 $\pm$ 0.19

Rabbit-anti-mouse thymocyte serum was prepared in rabbits according to the method of Levey and Medawar³. A pool of serum from eight rabbits was made after determining that each of the individual sera produced at least 90% suppression of the GVH reactivity of spleen cells 2 days after administration. The pooled ATS was inactivated by heating at 56° C for 2 h and absorbed four times with mouse erythrocytes (MRC) at a ratio of one part packed MRC to twenty parts ATS. At various intervals after the subcutaneous injection of ATS into the intrascapular region of Balb/cAnN (H-2^d) mice, GVH activity of various lymphoid tissues was determined in C57Bl/6N $\times$ Balb/cAnN (H-2^{b/d})F1 neonatal recipients using a modification⁹ of the spleen weight assay described by Simonson¹⁰. Thymus and spleen were rinsed in Hanks's balanced salt solution and cells gently teased into suspension. Nine days after grafting of cells, recipient litters were killed and the ratio of spleen weight to body weight was determined for both inoculated and control, uninoculated littermates. An index of spleen enlargement (spleen index) was obtained by dividing the spleen/body weight ratio of the injected animals by that

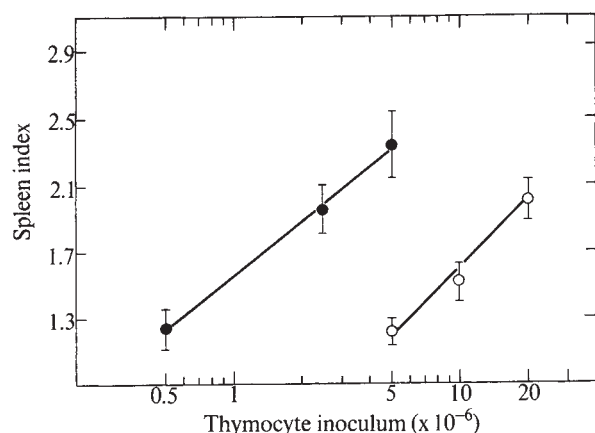


Fig. 1 Graft-vs-host activity of thymocytes obtained from donors given 0.25 ml. ATS subcutaneously 2 days earlier (●), compared with GVH activity of thymocytes obtained from mice given 0.25 ml. NRS subcutaneously 2 days previously (○). Vertical bars denote the limits of one standard error.

of their untreated littermates as previously described⁹. In several experiments the relative potency of two different cell suspensions was compared within the same recipient litter by dividing the litter into three groups: recipients of cell suspension 1, recipients of cell suspension 2 and control, uninoculated mice.

A reduction of at least 90% of splenic GVH activity was seen 48 hours after a single injection of 0.25 ml. ATS (Table 1). At the same time, however, a substantial increase in the GVH reactivity of thymus cells was noted. This increased GVH reactivity was present for the following 2 weeks and returned to pre-injection levels by the fourth week, when spleen GVH activity had returned to nearly normal levels (Table 1). It is possible to quantitate the change in GVH reactivity in both tissues after administration of ATS, assuming the activity curves for normal thymus and ATS-thymus are nearly parallel.

Table 2 Effect of Absorption of ATS with Thymocytes on Peripheral Suppressive Activity of ATS and its Enhancing Effect on Thymus GVH Activity

	Spleen index		% Normal reactivity*	
	Thymus	Spleen	Thymus	Spleen
0.5 ml. ATS	1.88	1.06	500	< 10
0.5 ml. ATA a	1.39	1.73	100	25
0.5 ml. NRS	1.26	2.45	N1	N1

* From previously published work^{12,13} and this study, using the parallel line assay.

Fig. 1 shows that this parallel relationship obtains at the time of peak reactivity of ATS-thymus; such parallel reactivity lines are seen with almost all tissues¹¹. The relative change in GVH activity in both tissues in the 4 weeks after a single injection of 0.25 ml. ATS is shown in Fig. 2. It is apparent that (1) the decrease in splenic GVH activity precedes by approximately 1 day the rise in thymic GVH activity and (2) this dose of ATS seems to have a strikingly reciprocal effect on the GVH activity of these two tissues.

It is unlikely that this effect represents simply the result of a steroid-mediated "stress" reaction and depletion of immunologically irrelevant thymocytes, as the cellularity of the thymus from mice treated with ATS did not differ significantly at any time from the control, uninjected mice (Table 1). To test whether this effect could be accounted for by a non-specific, adjuvant-like property of the rabbit serum, 2 ml. of the ATS was absorbed twice with 5×10^8 Balb/c thymocytes at room temperature for 10 min and compared with (1) ATS which

Table 3 Enhanced GVH Reactions on Mixing Thymocytes from Animals treated with ATS with Normal Thymocytes

ATS thymus* ($\times 10^6$)	NL thymus ($\times 10^6$)	NL PBL ($\times 10^6$)	Spleen index	Expected spleen index†
0.5	-	-	1.26 $\pm$ 0.08	-
-	5.0	-	1.29 $\pm$ 0.14	-
0.5	+ 5.0	-	2.28 $\pm$ 0.26	1.58
-	-	0.20	1.14 $\pm$ 0.06	-
-	5.0	+ 0.20	2.06 $\pm$ 0.18	1.58
0.5	-	+ 0.20	1.31 $\pm$ 0.19	1.58
5.0	-	-	2.37 $\pm$ 0.18	-
5.0	-	+ 0.20	1.84 $\pm$ 0.14	2.37

* Thymus from mice given 0.25 ml. ATS 2 days before killing⁶.

† By adding individual reactivity from the parallel line assay^{12,13}.

had been absorbed in parallel with 0.1 ml. packed mouse RBC and (2) normal rabbit serum (NRS). Absorption with thymocytes depleted ATS of both peripheral suppressive activity and its enhancing effect on thymus GVH activity (Table 2). Normal rabbit sera produced neither suppressive nor enhancing effects on either tissue.

Previous studies of cells which participate in the donor portion of GVH responses have demonstrated that two types of T lymphocytes can interact synergistically¹²⁻¹⁴. One cell type was found chiefly in the recirculating pool and appeared to amplify the response; the second was enriched in the thymus and spleen and appeared to provide precursors of

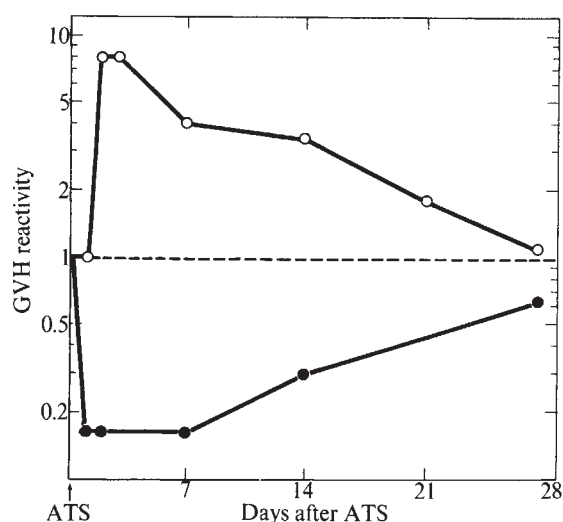


Fig. 2 Comparison of the relative change in GVH activity in thymus (○) and spleen (●) with time after a single subcutaneous injection of ATS (0.25 ml.). ---, Normal.

GVH effector cells. It was therefore of interest to test whether the increased thymic GVH activity could be attributed to an increase in the normally limiting amount of amplifier activity.

Mixtures of small numbers of thymocytes from animals 3 days after treatment with ATS with normal thymocytes produced GVH reactions that were substantially in excess of those predicted from the sum of their separate activities (Table 3). Moreover, unlike normal thymocytes, the activity of thymocytes treated with ATS was not significantly enhanced by the provision of small numbers of peripheral blood cells.

In fact, this combination produced a striking antagonistic effect.

These experiments indicate that administration of ATS to mice enhances the GVH activity of the thymus and that this effect is specifically related to the ability of ATS to recognize mouse lymphocytes. The finding is best explained by the idea that administration of ATS provokes an increased rate of differentiation of "immature", immunologically inert thymocytes to "mature" cells which have functional properties in GVH responses that are normally associated with peripheral recirculating T cells, such as the ability to interact synergistically with normal thymocytes in the production of GVH reactions (Table 3).

These experiments do not establish the mechanism responsible for enhanced thymus activity after an injection of ATS. One possibility is that the effect may represent a homeostatic mechanism in which increased intrathymic cell maturation is triggered by depletion of peripheral recirculating lymphocytes, perhaps by the products of ATS-induced lymphocyte destruction. This view receives some support from the observation that GVH hypoactivity in spleen preceded hyperactivity in thymus by as much as 24 h (Fig. 2) and is consistent with the subsequent reciprocal relationship of GVH activity in the two tissues over the following weeks. Alternatively, this effect may be the result of a direct effect of ATS on thymocytes *in situ*, resulting in the stimulation of thymocytes to divide and accelerated differentiation into mature cells. Although this possibility seems unlikely in view of the very poor penetration of labelled ATS into the thymus⁸, the demonstration that very low concentrations of some batches of ATS can stimulate lymphocytes to proliferate *in vitro*¹⁵ lends some support to this interpretation.

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1,25-Dihydroxycholecalciferol: Inhibition of Action in Organ-cultured Intestine by Actinomycin D and α -Amanitin

THE stimulatory action of vitamin D on intestinal calcium absorption has been much studied following the pioneering work of Nicolaysen¹ and several important features of the mechanism are fairly well established². Doubt concerning the chemical identity of the biologically active form of vitamin D has arisen, however, leading to extensive investigations of this aspect³⁻⁵. Two important proposals arose out of this work; first, vitamin D₃ (cholecalciferol) must be first converted to 1,25-dihydroxycholecalciferol (1,25-diOH-CC) (ref. 6) before it can act on the intestine; and second, 1,25-diOH-CC stimulates intestinal calcium transport directly by an actinomycin D-insensitive process⁶.

Organ culture of embryonic chick intestine was used to evaluate these proposals. Contrary to the first, vitamin D₃ itself in the culture medium was effective in inducing *de novo* synthesis of the intestinal calcium-binding protein (CaBP) and in stimulating radiocalcium uptake^{7,8} and mucosal to serosal transport^{9,10}. Here I report results that 1,25-diOH-CC stimulation of the intestinal calcium absorptive mechanism acts through new protein synthesis; both actinomycin D and α -amanitin prevented the response of the organ-cultured intestine to 1,25-diOH-CC.

Table 1 Inhibition of 1,25-diOH-CC Induction of CaBP and ⁴⁵Ca Uptake in Organ-cultured Chick Duodenum by Actinomycin D

Concentration in medium 1,25-diOH-CC (nM)	Actinomycin D (μ g ml. ⁻¹)	CaBP per 100 mg duo- denum (μ g)	⁴⁵ Ca uptake (% of zero 1,25-diOH- CC control)
0	0	0	100
0.65	0	2.73 $\pm$ 0.13	133.9 $\pm$ 3.9 *
0	2 (1.6 μ M)	0	100
0.65	2	0	95.3 $\pm$ 3.7

Values are the mean $\pm$ s.e. Duodena were cultured for 48 h in the presence of 5 mM Ca²⁺, 1,25-diOH-CC and actinomycin D in the culture medium. The tissues were analysed for CaBP content or transferred to a ⁴⁵Ca-containing buffer solution and incubated for 30 min at 37.5° C⁷. ⁴⁵Ca uptake was determined by liquid scintillation techniques on NCS (Amersham/Searle, Illinois) digests of the duodena. Student's *t* test was applied to the uptake data expressed as % dose ⁴⁵Ca accumulated/100 mg tissue. The uptake data were converted to "percentage of zero 1,25-diOH-CC control" for presentation.

* Treatments resulted in a significant increase in calcium uptake at the 1% level.

The entire duodena from four 20 day old chick embryos were opened and placed mucosa-side-up on a specially designed stainless steel grid⁸ inside a Petri dish containing serum-free McCoy's 5A (modified) medium, 2.5–5 mM calcium and 100 units nystatin ml.⁻¹. After 48 h in a humidified incubator continuously gassed with an air : O₂ : CO₂ mixture containing 5% CO₂ and 50% O₂, the duodena were either homogenized before CaBP measurement by radial immunoassay⁷ or subjected to measurement of radiocalcium uptake during a 30 min incubation in a buffer solution as described previously⁷.

Actinomycin D binds to deoxyguanosine residues in DNA, inhibiting DNA-directed RNA synthesis¹¹. Vitamin D and its metabolites localize in intestinal mucosal cell nuclei^{12,13}, and vitamin D stimulates DNA template activity¹⁴ and RNA synthesis^{15,16} in the intestinal mucosa, and the vitamin D enhancement of intestinal calcium transport^{17,18} is prevented by

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actinomycin D^{17,18}. Vitamin D induces *de novo* synthesis of CaBP^{7,19}, a protein probably involved in the intestinal calcium absorptive mechanism^{9,20}, which is inhibited *in vivo* by actinomycin D²¹.

When duodena were cultured in the presence of 1,25-diOH-CC (0.65 nM) and 5 mM calcium, CaBP was induced²², and there was enhanced uptake of ⁴⁵Ca (Table 1). (Stimulation of ⁴⁵Ca uptake in organ-cultured duodena can only be seen in response to a medium concentration of 0.65 nM 1,25-diOH-CC after 48 h incubation if calcium is between 2.5 and 5 mM⁸.) When 2 µg ml.⁻¹ (1.6 µM) actinomycin D was added to the culture medium, however, CaBP synthesis was completely

complete inhibition of 1,25-diOH-CC enhanced uptake of radiocalcium. This was not due to non-specific toxicity as inclusion of α-amanitin in the ⁴⁵Ca-containing buffer solution had no effect on 1,25-diOH-CC stimulation of radiocalcium uptake, supporting the conclusion that, like vitamin D₃, 1,25-diOH-CC acts on the intestinal calcium absorptive mechanism by stimulation of RNA and subsequent protein synthesis.

Table 2 Inhibition of 1,25-diOH-CC Induction of CaBP and ⁴⁵Ca Uptake in Organ-cultured Chick Duodenum by α-Amanitin

Concentration in medium 1,25-diOH-CC (nM)	α-Amanitin (µg ml. ⁻¹)	CaBP per 100 mg duo- denum (µg)	⁴⁵ Ca Uptake (% of zero 1,25-diOH- CC control)
0	0	0	100
0.65	0	3.8 ± 0.3	137 ± 4 *
0	0.5 (0.5 µM)	0	100
0.65	0.5	0	106 ± 3
0	0.5 (in ⁴⁵ Ca buffer only)	—	100
			136 ± 10 *
0.65	0.5 (in ⁴⁵ Ca buffer only)	—	

Medium (Ca²⁺) was 2.5 mM. See Table 1 for details.

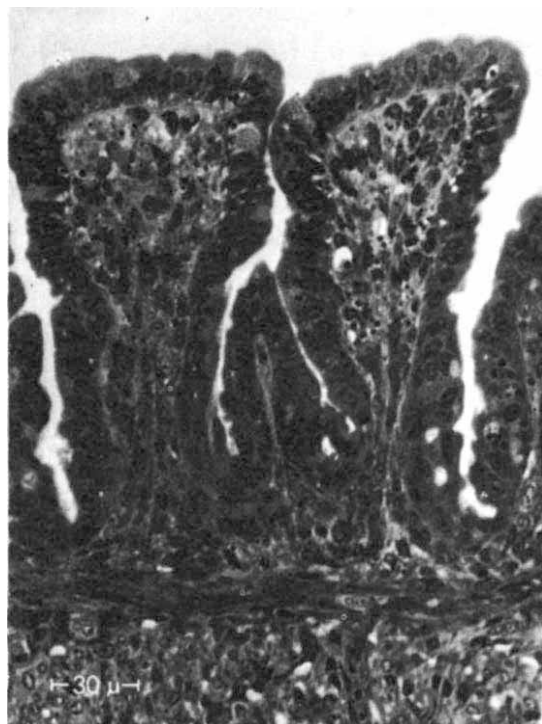


Fig. 1 Mucosal structure of embryonic chick intestine maintained in organ culture for 48 h in the presence of actinomycin D (2 µg ml.⁻¹) in the culture medium. Tissues were fixed in a glutaraldehyde buffer, dehydrated in methanol, embedded in glycol methacrylate, 1 µm sections prepared and stained with acid fuchsin-toluidine blue²³.

inhibited and ⁴⁵Ca uptake was not enhanced (Table 1). This was not the result of non-specific toxicity as total protein concentration of the mucosa was not reduced and mucosal structure was intact on histological examination²³ (Fig. 1). I suggest that, like vitamin D₃, 1,25-diOH-CC acts on intestinal calcium transport indirectly by stimulation of DNA dependent-RNA synthesis, and resultant CaBP and/or other protein synthesis, in the intestinal cell.

If intestinal RNA synthesis is essential to the action of 1,25-diOH-CC, then inhibition of DNA-directed RNA polymerase activity would also be expected to inhibit 1,25-diOH-CC induction of CaBP and stimulation of ⁴⁵Ca uptake as did actinomycin D. α-Amanitin, a cyclic octapeptide isolated from the green mushroom *Amanita phalloides*²⁴, specifically inhibits nucleoplasmic RNA-polymerase activity in isolated nuclei from sea urchin embryo, rat liver and calf thymus^{25,26}. When α-amanitin was included in the culture medium, the activity of 1,25-diOH-CC was inhibited (Table 2); first, CaBP synthesis was completely inhibited at a culture medium concentration of α-amanitin of 0.5 µM; and second, there was

To my belief, my results demonstrate α-amanitin inhibition of sterol induction of a specific protein in an *in vitro* system for the first time. Coupled with the findings of others that α-amanitin inhibited etiocholanolone induction of δ-aminolevulinatase in chick embryo liver *in ovo*²⁷, and cortisol induction of tyrosine transaminase in rat liver *in vivo*²⁸, it seems likely that α-amanitin will become important in the study of specific protein synthesis mechanisms in other systems.

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X-ray Photoelectron Studies of Magnesium Ions bound to the Cell Walls of Gram-positive Bacteria

TEICHOIC acids are strongly acidic polymers containing phosphate, which are present in all Gram-positive bacteria¹. They are found at two distinct locations in the cell envelope: associated with a glycolipid on the outer surface of the cytoplasmic membrane^{2,3} and covalently linked to the peptidoglycan of the cell wall³. The wall teichoic acid is responsible

cation environment for the activity of magnesium-dependent biosynthetic enzymes in the membrane^{5,6}. It is therefore important to identify the interactions involved in binding magnesium to teichoic acids in the bacterial envelope; although electrostatic interaction between the cations and negatively charged phosphate groups is a prominent feature, interactions with other components of the envelope cannot be ruled out. For example, the amount of D-alanine esterified to the wall teichoic acid of *Staphylococcus aureus* H and *Micrococcus* sp. 24 influences the capacity of these walls to bind Mg^{2+} (ref. 4).

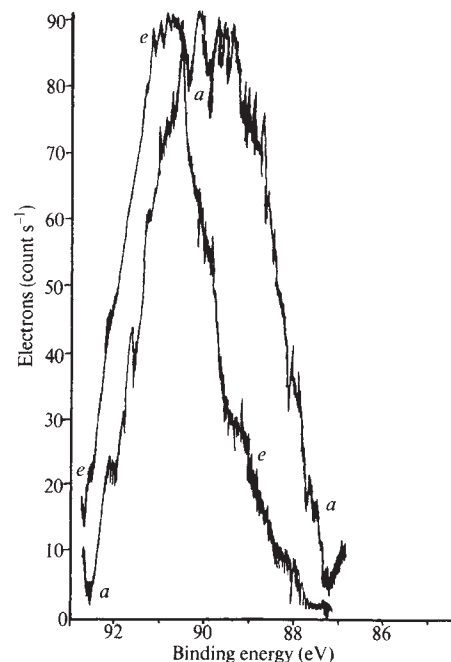


Fig. 1 X-ray photoelectron peaks for magnesium 2s electrons in combined sample of $MgCl_2$ and $MgHPO_4 \cdot 3H_2O$. a, With both salts exposed; b, with principally the chloride exposed.

Table 1 Composition of Preparations

	Phosphorus as % of total dry wt	Esterified alanine/ phosphate group	μeq Mg/ μmol phosphate
<i>Bacillus licheniformis</i> walls	1.33	0.50	0.36
<i>Bacillus licheniformis</i> wall teichoic acid from hydroxylamine-treated walls	12.1	0.02	0.95
<i>Lactobacillus plantarum</i> walls	3.0	0.75	0.30
<i>Lactobacillus plantarum</i> walls after treatment with hydroxylamine	2.8	0.25	0.44

for the ability of the cell walls of Gram-positive bacteria to bind divalent cations⁴ and the membrane teichoic acid mediates an interaction between magnesium ions, bound to the wall, and the cytoplasmic membrane, thus maintaining the optimum

Here we report a study by X-ray photoelectron spectroscopy (ESCA) of samples of cell wall. The samples were examined as powders mounted on double sided 'Sellotape' which was attached to the copper sample block, and were calibrated by reference to the gold 4f_{7/2} level electrons (binding energy 84.0 eV) from gold powder on the surface of the specimens. The calibration was done in a separate experiment because the gold electrons interfered with the magnesium 2s electrons; the cell wall nitrogen 1s peak was used as a reference. The spectra were studied using an AEI ES100 X-ray photoelectron spectrometer with Al Kα_{1,2} radiation. Overlapping peaks (electron count against binding energy) were deconvoluted using a Dupont curve analyser.

We have examined the walls of *Bacillus licheniformis* ATCC 9945 and *Lactobacillus plantarum* ATCC 10241 Mutant R1 (ref. 7). *B. licheniformis* was grown in a medium previously described⁵ and *L. plantarum* in the medium described by Douglas and Wolin⁷. Cells were disrupted with No. 11 Ballotini beads in a Braun cell disintegrator and the walls were recovered by centrifugation and washed with 0.9% NaCl and then water. The walls were heated at 100° C for 10 min in order to destroy autolytic enzymes and then washed repeatedly in water and freeze dried. Samples (50 mg) of walls were suspended in 10 ml. of 0.1 M $MgCl_2$, pH 6.0, for 1 h at room temperature, recovered by centrifugation at 20,000g for 15 min, washed four times with water to remove unbound cations and then freeze dried. Bound Mg^{2+} could be removed

from these preparations by suspension in 10 ml. of 0.05 M sodium EDTA, pH 6.5, for 30 min at room temperature, followed by centrifugation and washing. Alanine ester residues were removed from the teichoic acid in the walls by treatment with 0.4 M hydroxylamine (salt free)⁴. Wall teichoic acid was obtained by extraction with 10% trichloroacetic acid⁶

Table 2 Measured Binding Energies

	Binding energy (eV) *		
	Mg 2s	N 1s	P 2p
<i>Bacillus licheniformis</i> walls	90.8, 89.7	398.7	134.7
<i>Bacillus licheniformis</i> wall-teichoic acid from hydroxylamine-treated walls	88.8	399.9	133.3
<i>Lactobacillus plantarum</i> walls	90.6, 89.6	399.4	—
<i>Lactobacillus plantarum</i> walls after treatment with hydroxylamine	89.3	399.7	134.35
Magnesium chloride	90.7		
Magnesium phosphate (MgHPO ₄ ·3H ₂ O)	89.6		134.1

* Probably correct to ± 0.3 eV.

from walls of *B. licheniformis* which had been treated with hydroxylamine. It was converted to the magnesium salt by repeated passage through a column (1 cm $\times$ 10 cm) of 'Dowex' 50 resin in the Mg²⁺ form and freeze dried. Table 1 shows the compositions of these preparations and Table 2 the measured binding energies of the compounds. Alanine was determined

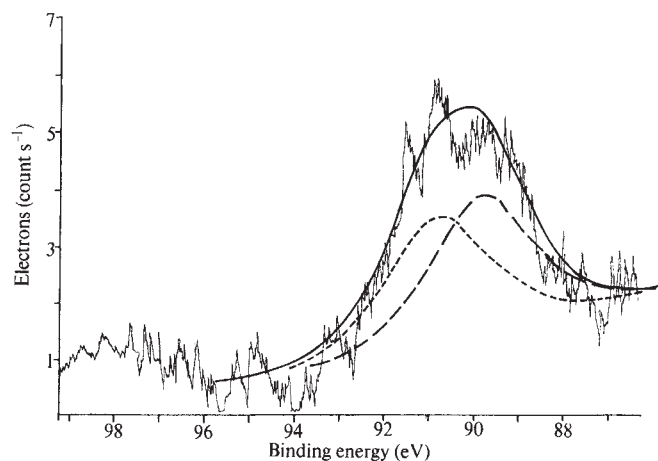


Fig. 2 X-ray photoelectron peak for magnesium 2s electrons in the cell wall of *Bacillus licheniformis*, showing the possible resolution of the broad peak into two component curves.

with ninhydrin⁸ after removal from the wall in 0.1 M NaOH, 37° C, 1 h.

In the charge potential model⁹ the binding energy of an electron can be expressed in terms of the charge on the atom (Q) and the Coulomb potential energy (V) of an electron due

to the other atoms of the molecule. Thus

$$\text{Binding energy} = \text{constant} \times Q + V + \text{constant}$$

In an ionic compound the term V includes the contributions from other ions and is therefore a Madelung term. Thus the difference (1.1 eV) in the binding energies of the 2s electrons of Mg²⁺ in magnesium phosphate and magnesium chloride is due to the difference in the Madelung terms for the two ionic compounds. Because this term represents interionic interactions in the ionic lattice we can treat a lower electron binding energy as an indication of a strong interaction of the Mg²⁺ with the anions in the crystal lattice. Because the accuracy of measurement of the binding energies is only claimed to be ± 0.3 eV, the small difference between the Mg²⁺ 2s electron binding energies in the two salts was demonstrated with a single rectangular sample one half of which was covered with MgCl₂ and the other half with MgHPO₄·3H₂O. The resulting broad peak is shown in Fig. 1a. When the sample was partly retracted to expose more of the chloride section and less of the phosphate a narrow peak at higher binding energy, due to the chloride Mg 2s electrons, with a small shoulder at lower binding energy, due to the phosphate Mg 2s electrons, were observed (Fig. 1e).

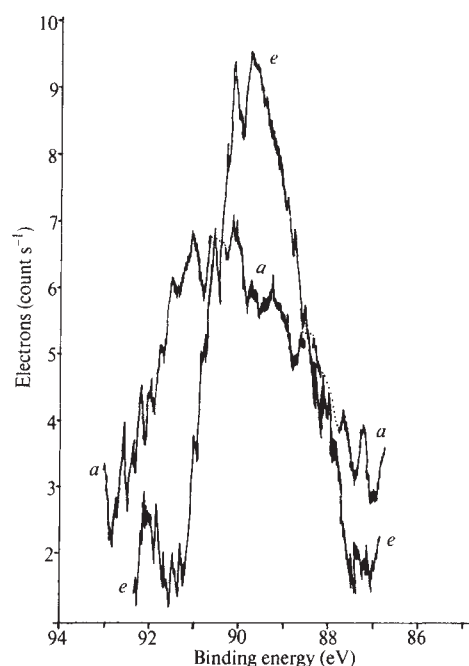


Fig. 3 X-ray photoelectron peaks for magnesium 2s electrons in the cell wall of *Lactobacillus plantarum* (a) containing esterified D-alanine and (b) with D-alanine removed.

The most significant result is the wide peak for Mg 2s electrons for both *B. licheniformis* (Fig. 2) and *L. plantarum* walls (Fig. 3a). This peak may be deconvoluted into two peaks on the basis of the measured width of the Mg 2s peak in magnesium phosphate. When the alanyl residues are removed from the *L. plantarum* samples there is a dramatic reduction of width to give the normal width expected for Mg 2s electrons (Fig. 3e). The measured binding energies thus indicate that the amino acid influences the ionic interactions of some of the magnesium ions. The interactions may be varied and complex, and the spectra may be properly interpreted in terms of many closely overlapping peaks rather than the two peaks shown. What is clear is that some of the mag-

nesium ions show a higher 2s electron-binding energy when the amino acid is present than when it is absent. The nitrogen binding energy in both cell walls comes in the region expected for an NH_2 group⁹.

Although the intensities in the X-ray photoelectron spectrum will be affected by differences in surface structure and sample state, in the case of the *L. plantarum* wall with and without the amino acids such differences may be small. The number of atoms of bound Mg^{2+} , estimated from the area under the Mg 2s peaks in Fig. 3, was reduced by 12% when the amino acids were present. This compares with a reduction of 25% measured by atomic absorption spectroscopy. Deconvolution of peak A in Fig. 3 into two component peaks indicated that 58% of the Mg^{2+} had the higher Mg 2s binding energy (90.6 eV) and 42% had the lower (89.6 eV).

Because, as we have already described, a higher Mg^{2+} electron binding energy represents a weaker ionic interaction between the Mg^{2+} and anions in the sample, the results indicate that in the presence of esterified alanine residues, a fraction of the Mg^{2+} is less strongly bound to the wall than that in the alanine-free walls, where it is known that the ionic interaction is dependent on phosphate groups⁴. The 2s electron binding energy in the weakly-bound Mg^{2+} corresponds to that in magnesium chloride, while the electron binding energy of the strongly-bound Mg^{2+} correlates with that of magnesium ions

which Mg^{2+} could bind strongly to two phosphates, and could thus produce a net movement of strongly bound Mg^{2+} on the polymer chain. This behaviour would be consistent with the known function of teichoic acids in both walls and membranes in producing and maintaining an appropriate divalent cationic environment⁶.

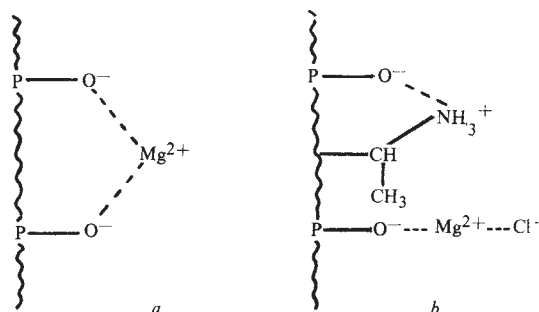


Fig. 5 Interaction of magnesium ions with wall teichoic acid.

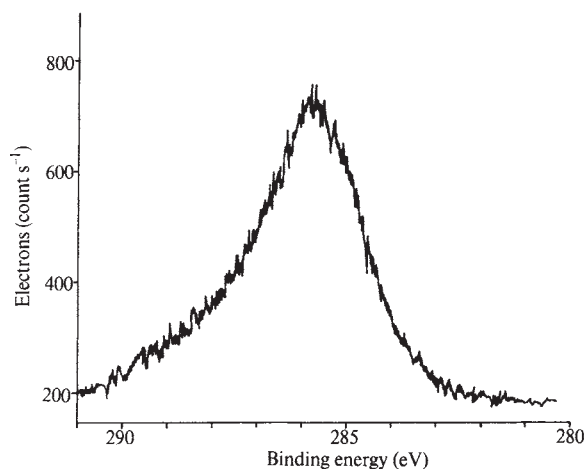


Fig. 4 X-ray photoelectron peak for carbon 1s electrons in the cell wall of *Bacillus licheniformis*.

in magnesium hydrogen phosphate, in which the Mg^{2+} interacts with two P-O^- groups. We suggest that the strongly bound magnesium ions interact with the wall teichoic acid as indicated in Fig. 5a, but that in the presence of alanine the $-\text{NH}_3^+$ of the amino acid may neutralize the charge on an adjacent phosphate group, thus permitting only one of the pair of phosphates to interact with Mg^{2+} as shown in Fig. 5b.

A consequence of this model is that in alanylated teichoic acid the weakly bound fraction of the magnesium ions might compete less successfully with other cations for the anionic sites on the polymer and the preparation is likely to contain monovalent cations as well as Mg^{2+} . This would account for the non-stoichiometric reduction of Mg^{2+} binding by walls which contain alanyl teichoic acid^{4,10} and which can be seen in Table 1.

It is possible that because of the flexibility of the polymer chain and the freedom of movement of the alanyl group a given alanyl residue may be capable of neutralizing one of a number of phosphate groups. A dynamic state in which the amino group alternates between several phosphates would produce changes in the positions on the polymer chain at

The carbon 1s electrons reveal other structural features of the cell wall. Thus this region for *B. licheniformis* (Fig. 4) shows an intense peak due to C-H carbons and pump oil carbon, together with peaks at higher binding energy due to the various C-O and C=O groups.

We thank Dr Julia Douglas for cell walls of *Lactobacillus plantarum*.

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The Brassins

A SERIES of papers¹⁻⁴ has described experiments purporting to show the existence of brassins from pollen of rape (*Brassica napus* Gaertn). It is claimed that they represent a novel group¹; that they consist of five active components; that they have a fatty nature^{1,2}; and that they have plant hormonal activity¹⁻⁴. We consider that the evidence presented in the four papers is inadequate to support any of these conclusions.

Our major criticism is that all the results reported were obtained with a mixture. No attempt was made to correlate quantitative measurement of any physical property with the intensity of a biological response; consequently there is no reason to relate any physical determination with the active molecule(s). No two measurements need necessarily relate to the same component of the mixture. Even the simple and fundamental separation of the extract into ether-soluble acidic, basic and neutral compounds was not attempted.

To determine the constitution of the brassins a fraction was separated¹ from an ether extract of dry rape pollen by TLC of the crude extract on silica gel, developing with benzene-methanol-acetic acid (45 : 8 : 4); the active fraction (R_F 0.35 to 0.45) was then rechromatographed with isopropyl ether-acetic acid (19 : 1). The activity remained on the origin and the eluate was termed brassins.

The crude brassin fraction was subjected to NMR analysis in $CDCl_3$ solution¹. No integration or multiplicity of observed resonances was given and yet from a few undefined signals in the δ 1.26 to 2.00 region, a "signal" at δ 3.8 ("CH₂ attached to oxygen"), and a "multiplicity character" at δ 5.3 ("olefinic protons") the authors decide that this crude fraction is fatty acid ester material and suggest a glyceride structure. This information is insufficient to warrant these structural conclusions. The active substance(s) may have been so dilute that it (they) escaped detection by NMR spectroscopy. In the NMR spectrum of tristearin⁵ in $CDCl_3$ the glyceryl methylene and methine protons comprise an A_2B_2C system with resonances centred around δ 4.2 and δ 5.3 respectively. No saponification of these "fatty acid esters" was attempted to support the authors' structural hypotheses.

The brassin fraction was not tested in any of the familiar bioassays which are reasonably specific for auxins (oat coleoptile elongation), gibberellins (growth stimulation of dwarf maize or α -amylase synthesis in barley aleurone) and cytokinins (tobacco pith callus growth), therefore a contribution of these hormones to the activity of the brassin fraction has not been excluded.

Combined GLC-MS of the crude brassin fraction was carried out and "several components" were observed and some features of their mass spectra were recorded¹. No attempt was made to correlate the biological activity with observed GLC peaks and no fractions were collected and tested. Apparently no derivatives were prepared (for example, by methylation or trimethylsilylation of acidic components) before GLC to render volatile any involatile and possibly active components and so several may have escaped detection.

'Sephadex LH-20' gel chromatography, monitored by ultraviolet absorption at 254 nm, gave five peaks, all containing active material; but the conclusion that five active substances were present is not justified. These five ultraviolet absorbing fractions could overlie a spread of one active material—it is necessary to demonstrate inactive areas between peaks of biological activity before the presence of more than one active component can be claimed; even then the possibility of an inhibitor would have to be excluded.

The brassins were originally extracted from rape pollen but no experiments have been carried out to determine their effect on the physiology of rape pollen, or even on a rape plant. Consequently there is no direct evidence for their having a hormonal role *in vivo*. The extract of rape pollen undoubtedly accelerates the growth of bean (*Phaseolus vulgaris*) test plants, but the activity may be mainly attributable to a gibberellin

because the elongation of internodes is a characteristic gibberellin response. Gibberellin activity occurs in high concentrations in ripe anthers (4 μ g GA₃ equivalents g⁻¹ dry weight in *Vitis*)⁶. The dual cell division and cell elongation response of bean internodes to brassins was reported to be histologically different from that induced by GA₃, because GA₃ did not induce cell division³. GA₃, has, however, been reported to stimulate cell division and elongation in petioles of strawberry⁷, stems of *Hyoscyamus*⁸ and even of internodal cells of bean (*P. vulgaris*, c. var. Blue Lake)^{9,10}, so the claim of "a new kind of growth effect"³ is unfounded. The greater responsiveness of small (slow-growing) bean plants to applications of brassins is similar to the more pronounced effects of gibberellins on dwarf plants¹¹; furthermore, the presence of more open flowers on brassin-treated bean plants⁴ than on controls is also a typical response to gibberellins (see illustration in refs. 12, 13 or 14). In the light of the foregoing comments, and those of Stowe and Dott¹⁵, it is idle to criticize the postulated roles of the brassins, concepts such as "alpha hormone"⁴ and the suggestion that "... brassins seem to function early in the development of the embryo, ..."; suffice it to say that the only data reported from experiments which involved embryos were the weights of ripe bean seeds produced on plants treated with brassins. We consider that the claims advanced for brassins being a novel complex of fatty plant hormones are premature and unjustified on the basis of work published so far.

Note added in proof. Since this manuscript was completed Mandava and Mitchell¹⁶ have published a further paper on the "brassins".

They conclude from NMR and mass spectroscopy data of derivatives that "brassins" are glucose esters of fatty acids; this conclusion is inconsistent with NMR data given and earlier NMR and UV data¹ cited as evidence for a glyceride structure. The authors have not correlated biological activity with any physical measurement, consequently the attribution of "brassin" activity to any specific substance is unjustified.

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BOOK REVIEWS

Mythical Quagmire

From Honey to Ashes: an Introduction to a Science of Mythology. By Claude Lévi-Strauss. Translated from the French by John and Doreen Weightman. Pp. 512. (Jonathan Cape: London, February 1973.) £6.50.

THIS is the translation of *Du Miel aux Cendres*, the second volume in Claude Lévi-Strauss's magnum opus, *Les Mythologiques*. We are promised English editions of the third and fourth volumes in due course, and we can only admire the stamina of the translators in tackling this particular *Hydra*. At the same time, however, we might wonder whether, when the monster is finished, it will have been worth the effort.

The first volume in this *Science of Mythology* (as the term *Mythologique* has been unfortunately translated), *The Raw and the Cooked* (reviewed *Nature*, 226, 875; 1970), is concerned with the exploration of the opposition of the empirical categories raw and cooked food as they are expressed in South American Indian mythology. This second volume continues this examination of the use of culinary categories although this fact is less easily concluded from the title. Here the opposition is between honey and tobacco, which, according to Lévi-Strauss, can be stated in the following culinary terms: honey comes from nature raw and ready for consumption and because it needs no preparation it belongs to the "hither side" of cooking, it is infra-culinary. On the other hand, tobacco is consumed neither raw nor cooked but its consumption involves reducing it to ashes and thus belongs "beyond" cooking, it is super-culinary. This initial opposition later requires modification because it only represents the natural properties of the two substances and their characters may be changed by cultural processes such as the fermentation of honey or the extraction of tobacco juice. Once again the material in which these oppositions and their meanings are explored is South American mythology; a large proportion of the 187 myths used in the first volume are re-examined together with 166 new ones.

It is virtually impossible to give any coherent picture of the content of this book, and for the purpose of this review it will suffice to note the aim and the conclusion. The aim is nothing less than to reveal the architecture of the mind as it is represented by mythic thought. The author claims that in this volume he has made an important step in that direction because his analyses have shown that the myths contain not simply a logic of qualities (the oppositions of raw/cooked, wet/dry, and so on) but also a logic of forms (the oppositions of empty/full, internal/external, and so on). He argues that the analysis has reached the point "where mythic thought transcends itself and, going beyond images retaining some relationship with concrete experience, operates in a world of concepts which have been released from any such obligation, and combine with each other in free association". This is the point, it is claimed, at which mythology gives way to philosophy and the existence of the latter is the necessary precondition of scientific thought. In other words, latent in mythic thought is scientific thought awaiting the right time and conditions to emerge. The unity of the human mind is assured.

What is the general reader to make of it? Probably not very much. He is assured that there is no need for him to read the first volume in order to understand the second because "the world of mythology is round . . . the reader can begin where he chooses and yet be sure of completing the course". While one must take the author's view on this characteristic of his work, it is a little difficult to see how the reader new to these works could understand what is going on without reading at least the overture to *The Raw and the Cooked*. (In fact, in the foreword to the third volume it is admitted that the world of mythology is not quite as round as had been assumed.) A second difficulty is the weight of ethnographic material which the reader is asked to carry in his mind. The load has become insupportable, and although one must admire the author for achieving the control he

has of such a mass of evidence (zoological, botanical, and cosmological as well as ethnographical) it is only too easy to fault him. Some of his admirers claim that such criticisms are small-minded and unimportant compared with the grandeur of his aims. While the specialized reader may be dismayed by Lévi-Strauss's cavalier attitude towards and interpretation of the ethnography, however, he cannot fail to be impressed, perhaps even excited, by the quite extraordinarily imaginative associations and connexions which he makes. For those who are sceptical about these, it might be pointed out that recent field research in South America has demonstrated that these sorts of oppositions and analogies actually exist in the consciousness of Amerindians who seem to elaborate them as an intellectual exercise. This raises the further questions whether it would not have been easier for Lévi-Strauss to have achieved the same result more reliably through intensive fieldwork, or whether it has only been as a result of his intuitions that this mythological thought has been discovered to exist.

But a more serious difficulty for any reader does not lie simply in the mass of material, but in the nature of the analytical framework. It might be hoped that after over 800 pages of analyses some analytical tools and concepts would have taken on some more rigorous nature. There is no sign that Lévi-Strauss has made any attempt to produce such definitions, and the analytical procedures remain as vague and pliable as they are at the beginning of volume 1. In fact, the situation is worse because suddenly on page 249 he reintroduces an algebraic formula (of doubtful logical validity), first seen in his *The Structural Study of Myth* (1955) and not since, and declares that it has never ceased to guide him. The central notions of transformation and opposition still lack definition, terms such as armature and code still fail to convey any sure sense, and the result is that the reader, lacking any certain methodological path, tends to lose his way in

the quagmire of myths. The work is studded with claims that this has been demonstrated, that has been proved, but the conclusions that are drawn do not seem necessarily to arise from the analyses. In the end there is just frustration.

PETER RIVIERE

Psychological Approaches

Humanistic Psychology and the Research Tradition: Their Several Virtues. By I. L. Child. Pp. vi+213. (John Wiley: New York and London, January 1973.) £4.15.

PROFESSOR CHILD was the author of *Italian or American*, a classic study of the conflicts created in children by discrepancy between the values and style of life of their home and those of the outside society. Although now 30 years old, this study should still demand for its author a respectful reading of any fresh work. The present book, moreover, is particularly sympathetic in approach: it attempts to reconcile different attitudes and points of view about human beings, to extract constructively the best of each, and to reconcile rather than oppose. By "humanistic psychology", Professor Child means those currents of thought in which man is viewed somewhat as he normally sees himself—as a person rather than only as an animal or a machine. The "research tradition", on the other hand, is that of systematic factual enquiry, in which the ideas and methods of study may be highly impersonal in their pursuit of objectivity. The technique of reconciliation which the book uses is to review researches conducted in the empirical tradition, but whose results and interpretation would favour the humanistic attitude. Thus there are chapters on moral development, on the psychology of art, on hypnosis, on cognitive dissonance, on extrasensory perception (ESP), and on clinical work in schizophrenia, psychotherapy, and behaviour therapy. In each of these areas, the choice of investigations is interesting, and they are very well described. Few of us can fail to be interested by evidence for the incidence of ESP being greater in people who believe in it, by agreement between art experts from very different societies on the relative merits of different artists; or by an apparent regression of ethical judgment in some people of university age as they work through to a higher level of adjustment.

Professor Child's praise and criticism of each approach, furthermore, are sound and well judged. In claiming that their virtues might be combined, and their vices abandoned, he is on very sound ground. The book ought there-

fore to be consulted by anybody who would like to achieve this end.

And yet, this smooth reconciliation does conceal genuine abrasive disagreements, which an upholder of the research tradition must emphasize. In several places, the humanistic conclusion from one study contradicts that from another. Moral development is supposed to be independent of the society in which it occurs: and yet the differences in behaviour in prisons, mental hospitals, and everyday life are to be ascribed to the different environments rather than to the individual. The best judges of artistic merit are those who reject the social pressure of others around them: and yet certain techniques derived from the Esalen Institute are suggested to increase hypnotizability, and this is regarded as desirable. A finding that those schizophrenics who report themselves as highly disturbed in a questionnaire also do very badly by objective tests of performance is interpreted as meaning that both scores are the common result of faking to secure a stay in a congenial hospital environment. This interpretation seems highly questionable, and it is not clear that it is more humane than believing what the patient says. Lastly, this book deals only with conclusions about personal choice and self control of which the humanistic thinker may approve: a true reconciliation of the two approaches will need also to take into account the massive evidence which the research tradition has provided of the distortions, limitations, and constraints which the machinery of ourselves and our society may place upon that choice. The commonsense concept of personality may be true enough as far as it goes; and yet it is a problem rather than a solution. DONALD E. BROADBENT

Machine Botany

A Computer-Mapped Flora: A Study of the County of Warwickshire. With a section on Bryophytes by T. Laffin, and contributed chapters by F. W. Shotton, H. Thorpe and G. T. Warwick. By D. A. Cadbury, J. G. Hawkes and R. C. Readett. Pp. ix+768. (Academic: London and New York, December 1971.) £10; \$30.

IN 1949 it was decided that, as so many changes had taken place in habitats and plant distribution in the county since the publication of Bagnall's *Flora of Warwickshire* (1891), a new survey was needed. A single kilometre square selected at random from each block of four (tetrad) was adopted as the basis for detailed study, and recorders listed habitats and frequencies of species on standard recording forms. This information was eventually conveyed to punched tape for processing by a com-

puter. An incremental graph plotter and a line printer produced maps of vascular plants and bryophytes.

The maps occupy 420 pages and those for vascular plants are of two kinds. The first, covering 718 species, are complex and give three main pieces of information: presence or absence of the particular species; the major habitats in which the species was recorded (by the use of nine different superimposed symbols); and an approximate idea of frequency of the species in each habitat. The second, covering fifty-nine less common species, are simpler, and indicate only presence and frequency. Two frequency categories—abundant to frequent, and occasional to rare—are distinguished in the maps, but unfortunately the authors give no quantitative definitions of these terms, so it is left to the reader's imagination (as it was probably left to the ingeniousness of the recorders) to decide what they mean. The distributions of 184 different bryophytes in a fruiting or non-fruiting condition are also mapped. A pocket at the end of the book contains twelve overlay maps which show, besides physical and meteorological features, woods, parklands, heaths and commons.

The check lists accompanying the maps include information on first Warwickshire records, habitats, and occurrence in neighbouring counties. There are keys to the local species of *Rubus fruticosus* agg., *Salix* (including hybrids), *Arctium*, *Hieracium*, and *Bromus*. Chapters on the physical background, geology and soils (with an excellent map showing soil types), historical geography (explaining the changing patterns of natural vegetation in the county from prehistoric times to the present day), and habitat studies, complete the flora.

A shortcoming of the work is the recording in but one square kilometre out of every four. Although it ensures an even survey the result is that only one-quarter of the county is covered, and in spite of the inclusion of records from some squares chosen non-randomly, about 65 per cent of the county is left unworked. Is this the reason why so many species known to the old Warwickshire botanists are "not recorded in the present survey"? How much more satisfying if all squares had been surveyed: but to have done this with the thoroughness of the present work would have been an impossible task. So we should consider *A Computer-Mapped Flora* as an academic exercise expertly planned and accomplished, rather than as a definitive county flora. It is the first flora in which habitat data have been mapped by a computer, and we are grateful to the authors for introducing us to an entirely new approach to vegetational research.

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